RNI. No. DEL/1998/4626 ISSN 0973-3507

Vol. II No. 29 DEC 2020

Universities' Journal of Phytochemistry and Ayurvedic Heights

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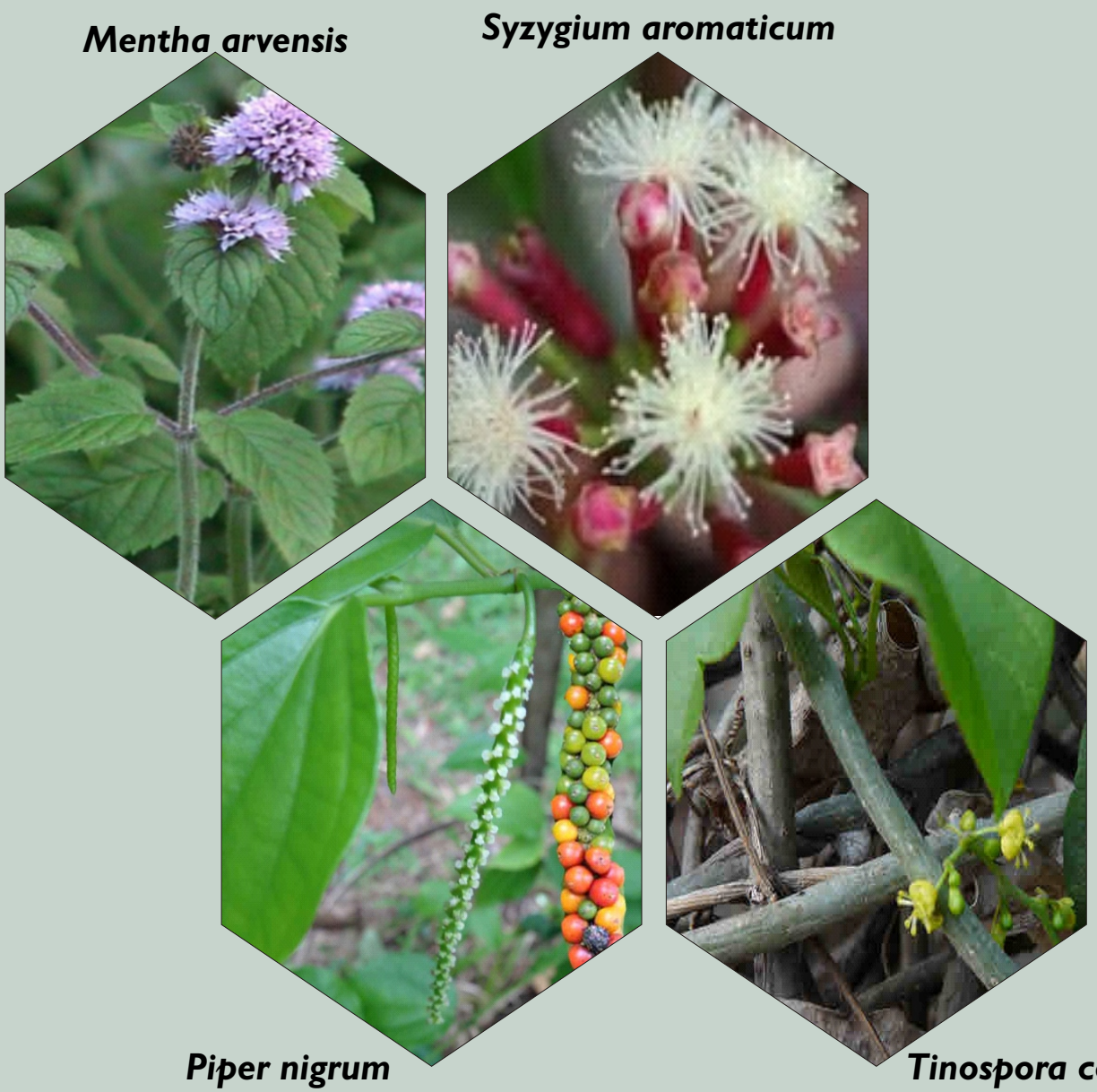

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Editorial

The year 2020 is called the year of Pandemic which affected the whole world.This **COVID-19 pandemic**, also known as the **coronavirus pandemic**, is an ongoing pandemic of coronavirus disease 2019 (COVID-19) first identified in December 2019 in Wuhan, China. The World Health Organization declared the outbreak a Public Health Emergency of International Concern in January 2020 and a pandemic in March 2020. As of December 2020, more than 70 million cases have been confirmed, with more than 1.5 million global deaths attributed to COVID-19 in which India crosses 139000 deaths.
This pandemic has made the entire world realise the need to reimagine supply chains. India imported 70 per cent of its API from China. The Govt of India has announced in Apr'20, INR 10000 crore of investments to incentivise production of APIs in India. Therefore Pharma students should note the silver lining of their career in Pharma stream which is expected to boost the business of Indian Pharma Industry. This exclude the expected Indian vaccine which is being indigenously developed by Bharat Biotech in collaboration with the ICMR and SII is in Phase 3 of trials.

The responses have caused global social and economic disruption, including the largest global recession . It has led to the postponement or cancellation of events, widespread supply shortages exacerbated by panic buying, agricultural disruption and food shortages, Educational institutions have been partially or fully closed or working on new order of leading life by E education, E business, work from home and webinar etc. We all know that prevention is better than cure it will be good to take preventive measures which boost our immunity in these times. Having a vaccine, alongside better treatments, is "the" exit strategy. The big breakthrough came when Pfizer/BioNTech **published its first results in November.** They showed the vaccine is up to 95% effective. On 2 December, the UK became the first country in the world to approve the Pfizer/BioNTech corona virus vaccine for widespread use.
The WHO Situation Report shows that India has one of the lowest deaths due to COVID-19 per million populations. India's cases of death per million population is 14.27 while the global average is more than it's four times, at 68.29 and Bill Gates says we may have back to normal by the year 2020.

In the wake of the Covid 19 outbreak, entire mankind across the globe is suffering and trying to enhance the body's natural defence system (immunity) plays an important role in maintaining optimum health Where herbs have played a pivotal role in controlling this pandemic. Fortunately Indian food which contain culinary herbs and act as an immunobooster being a Neutraceutical have also enhanced immunity to its countries at large.
Ayurveda, the 5000 years old system propagates the gifts of nature in maintaining health therefore, Ministry of AYUSH recommends Spices like Haldi (Turmeric), Jeera (Cumin), Dhaniya (Coriander) and Lahsun (Garlic) in cooking. Drink herbal tea / decoction (Kadha) made from Tulsi (Basil), Dalchini (Cinnamon), Kalimirch (Black pepper), Shunthi (Dry Ginger) and Munakka (Raisin) and Golden Milk- Half tea spoon Haldi (turmeric) powder in 150 ml hot milk - once a day.

Eating excessive culinary herbs/food/vegetables and condiments are harmful leads to gastric problems, acidity and stomach discomforts, liver toxicity and low blood pressure, therefore it should be taken with utmost care and in recommended doses unless one may get the adverse effects instead of benefits. **Neutraceutical** also play a very important **role** in boosting **immunity** without harming body's natural defence mechanism. Examples of important **Neutraceutical** such as Amla for vitamin C, Almonds and pumpkins for zinc, and broccoli and cabbage for selenium, they **boost** the **immunity** of human body system.

UJPAH June 2020 issue was released by Honourable Minister of HRD Dr. Ramesh Pokhriyal Nishank. He is a wonderful person his ideas definitely gave encouragements to the participants for research with available resources on Indian Herbs. I am fortunate to be in touch with him since long and very thankful to him to become a part of that ceremony of releasing of Universities' Journal of Phytochemistry and Ayurvedic Heights June 2020 issue.

This issue of UJPAH is again whole heartedly devoted to Ayurvedic immunity booster herbs to maintain the immunity in this Covid era of crisis.

My best wishes to all those scientists, Research scholars, students and teachers who contributed for bringing out this issue and I also express my sincere gratitude to all board members who make this issue a memorable for science fraternity of the Uttarakhand and the people of science at large. Last but not least I express my thanks for all those who have sent messages for this December 2020 issue of the journal.

Dr. S. Farooq
Chief Editor

Contents

Physico-Chemical Studies of Seed Oil of *Prinsepia utilis* Royle (bhekal) Grown in Chakrata Region and Its Comparison With Conventional Food Oils

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DOI 10.51129/ujpah-2020-29-2(1)

Abstract-Oil and fats are important component of human dietary system. Increase in demand and different applications of oil foster the search for vegetable and seed oil of high quality. In India several regions have developed specific preference for edible oil locally available. Several pockets in Garhwal Himalayan region, people give preference to some wildly growing species. *Prinsepia utilis* (bhekal) is one of that kind. This paper deals with the investigation of physicochemical characteristics, fatty acids composition and mineral content seed oil of bhekal (*P. utilis*). Mature seeds were collected from Chakrata forest division, Chakrata, Uttarakhand, India and oil was extracted by conventional method. Extracted oil was yellow in colour, tasteless, odour less, soluble in benzene, pet-ether and slightly soluble in acetone. Its physico-chemical and proximate values were determined by appropriate lab method. The elements present in seed oil were also determined which reflect that oil is rich in Iron, Sodium, Potassium and appropriate range of Potassium, Zinc, Calcium and Magnesium which make it suitable for edible consumption and commercial purposes. The composition of fatty oil was determined by Gas Chromatography (GCMS). From GCMS analysis the main constituents of oil such as Myristic acid (13.10%), Palmitic acid (10.20%), Stearic acid (21.90%) and Oleic acid (4.50%) were determined. Nutritional and physico-chemical properties of the *Prinsepia utilis* oil and their comparison with other food grade oil suggested that oil is suitable for use in domestic and for the industrial uses.

Keywords: *Prinsepia utilis*, Edible oil, Proximate value, Fatty acid composition, Mineral content.

Introduction

A variety of plants produce their reserve food in the form of oil which stored either in seed or in their fruit. The seed that contain oils are called "oil seeds" and these oil seeds are receiving growing interest due to their high concentration of bioactive lipid components which have shown various health benefits and constantly consumed in most developing and under developed countries by both man and animals. A number of seed oils have been characterized for the identification of several fatty acids of nutritional and nutraceutical importance but the vast majority have not been adequately evaluated. Increase in demand and different applications of oil foster the search for vegetable and seed oil of high quality such as *Prinsepia utilis*. Royle (Family Rosaceae) seed. It is commonly known as Bhekal, Bekhali, Bekoi, Jhatlu etc. It is a moderate sized underutilized deciduous shrub widely distributed in Himalayas at 1000 - 3000 msl., occurring frequently in open, sunny and dry places. Flowers season is from the month of December-January, while fruit ripining occurs during the month of April and June. Fruit are single seeded, oblong in shape and have fleshy edible Mesocarp. Bhekal seed oil is preferably used in remote areas of Garhwal Himalayan but very scanty information is available on its proximate values and on the fatty acid compositions.

Material and Methods

Collection of plant material and extraction of oil

Fully mature fruit of *P.utilis* were collected during the month of May-June from Chakrata Forest Division, Uttarakhand, India which is at an altitude of 2700 m ($30^0$37'00.25" N, $77^0$52'28.09" E). The plant was identified by the Taxonomist at systematic Borany Division, Forest Research Institute, Dehradun, India. A voucher specimen has been deposited and received Accession no. 172708. The collected seeds were dried and grounded to powdered via using a mechanical grinder after the removal of seed coat (AOCS, 2001). The fatty oil was extracted with petroleum ether solvent (b.p. 40-60 $^{\circ}$C) by using soxhlet apparatus.

Determination of Physio-chemical properties of seed oil

The fatty oil was accessed for the determination of proximate and physicochemical properties. Seed oil colour, taste, smell, sedimentation, physical state at normal room temperature were subject of general observation, whereas refractive index was determined at 25°C with Abbey Refractometer. The seed oil was assessed for various physico-chemical properties. Standard methods described by Association of Official Analytical Chemists (AOAC,1990) were used for the determination of yield per cent, moisture content, ash, crude fibre and free fatty acids (FFA) contents of the fatty oil. Iodine value was determined by Wij's method (AOAC, 1990). Saponification values, unsaponifiable matter content and acid value of the oil sample was determined by the procedures of Egan *et al*. (1981). Per cent Protein was determined using micro-Kjeldhal method as adopted by Allen and Quarmby (1989). Carbohydrate content was determined by colorimetric method described by Allen and Quarmby, 1989

Determination of mineral content of oil

The metal composition Zinc, Iron, Copper of theoil were determined by using an Atomic Absorption Spectrophotometer (Model Varian AT2049/France), after acid digestion. Total calcium and magnesium were determined by complexometric titration with 0.1M EDTA, by using blue dye Erichome Black T (Erio T) was used as indicator and calculated. Phosphorus was determined by the precipitation of phosphorus in the form of phosphomolybdate by using the reagent ammonium molybdate. Precipitate was filtered from asbestos, then residue obtained was taken in conical flask and dissolved in 0.1M NaOH and titrate with 0.1M HCl by using indicator Phenolphthalein. Sodium and potassium were determined by flame photometer.

Determination of fatty acid composition of oil by GC-MS

Fatty acid composition of fatty oil was determined by Gas Chromatography and Mass spectrophotometer. 0.2g of oil sample were weighed into 250ml conical flask and methylated with 6ml of sodium methyloxide.

Results and Discussion

The results obtained in the present study quantified the physico-chemical properties, mineral content and fatty acids composition of bhekal seed oil. The results obtained are given in tables under each of its respective topics as follows.

Determination of physico-chemical and proximate value of oil

Physico-chemical properties were determined by following standard methods. The oil extracted from bhekal is yellow in color, having pleasant odour, tasteless and liquid at room temperature (25±5 °C) but below normal room temperature it is semi-liquid or get solidified in its crude state. The fatty oil yield was 51.00% with very low amount of moisture percentage (1.20%) which is free from sedimentation. Data of all physico-chemical properties are being given in table -1.

Table-1 Physico-chemical properties of *P. utilis* seed oil.

S. No.	Physico -chemical properties	Results
1	Colour	Pale yellow
2	Taste	Neutral, free from bitter and after test
3	Smell	Pleasant
4	Sedimentation	Free from sedimentation
5	State at room temperature	Liquid
6	Refractive index (at 25 ^{o}C)	1.36
7	Specific gravity (g/cm^{3})	0.84
8	Yield (%)	51
9	Moisture (%)	1.20
10	Protein (%)	0
11	Fiber (%)	0
12	Carbohydrate (%)	0
13	Acid value (mg/KOH/g)	3.97
14	Iodine value	142
15	Saponification values (mgKOH/g)	220
16	Unsaponifiable matter (%w/w)	0.02
17	Free fatty acid (%)	13.90
18	Total saturated (%)	21.80
19	Total unsaturated (%)	34.60

Determination of mineral content of Bhekal oil

The level of phosphorus, calcium, magnesium, sodium, potassium and manganese were found in good amount in seed oil, while the oil has much lower amount of iron, copper and zinc (Table-2) fall within the range of values obtained for some vegetable oils.

Table-2 Mineral content seed oil (mg/100g) of *P. Utilis*

S. No	**Parameters**	**Results** (mg/100g)
1	Phosphorus	428
2	Calcium	312
3	Magnesium	3.21
4	Sodium	0.48
5	Potassium	1.02
6	Iron	3.90
7	Copper	1.32
8	Zinc	3.19

Determination of composition of bhekal oil by GC-MS

GC-MS analysis of fatty acids showed that the main constituents of oil is Myristic acid, Palmitic acid, Stearic acid and Oleic acid (Table-3).

Table-3 Fatty acid composition of *P. utilis* seed oil(mg/100g)

S. No	**Parameters**	**Results**
1	Myristic acid	13.10
2	Palmitic acid	10.20
3	Stearic acid	21.90
4	Oleic acid	4.50

A comparison of bhekal oil properties was also done with the previously reported properties of some conventionally used oils (CUOs), for food and other purposes by Gopalan et al., 1971; Anonymous, 2001 and Bachheti et al,2015. Bhekal oil is existing in liquid state at room temperature as all other CUOs but it gets solidified or in semi-solid status at below room temperature as coconut oil. The physical colour of bhekal oil is also in colour range of CUOs which varies from colorless, yellow to dark brown. The proximate chemical composition and physical attributes of the nine different CUOs and bhekal oil are given in Table-4 and Table-5.

Table-4 Proximate composition of some conventional oil and bhekal oil

Species	**Moisture (%)**	**Yield (%)**	**Protein (%)**	**Fibre (%)**	**Carbohydrate (%)**
Soybean oil (*Sojamax*)	8.1	19.5	43.1	3.7	20.9
Mustard seed oil (*Brassica compestris*)	8.5	39.7	20.0	1.8	23.8
Ground nut oil (*Arachis hypogoes*)	3.0	40.1	25.3	3.1	26.1
Cotton seed oil (*Gossypium*)	9.9	19.5	19.4	22.6	23.9
Linseed oil (*Linum usitatissium*)	6.5	37.1	20.3	4.8	28.9
Sunflower oil (*Helianthus annus*)	5.5	52.1	19.8	1.0	17.9
Coconut oil (*Cocos mucifera*)	4.2	39.0	23.9	10.9	17.1
Almond seed oil (*Prunus arnygdalyus*)	5.2	58.9	20.8	1.7	10.5
Wild apricot (*Prunus armeniaca*)	6.8	44.3	31.8	1.94	15.6
Bhekal (*Prinsepia utilis*)	**1.0**	**51.0**	**0.0**	**0.0**	**0.0**

Table-5 Physical attributes of some conventional seed oil and bhekal oil

Species	State at room temperature	Colour	Refractive index (40 ^{o}C)	Specific gravity at 25 ^{o}C
Soybean oil (*Sojamax*)	Liquid	Yellow	1.46	0.91
Mustard seed oil (*Brassica compestris*)	Liquid	Brownish yellow	1.46	0.90
Ground nut oil (*Arachis hypogoes*)	Semi solid	Colour less	1.46	0.91
Cotton seed oil (*Gossypium*)	Liquid	Yellow	1.46	0.91
Linseed oil (*Linumusitatissium*)	Liquid	Colour less	1.47	0.93
Sunflower oil (*Helianthus annus*)	Liquid	Pale yellow	1.46	0.91
Coconut oil (*Cocosnucifera*)	Semi liquid	Colour less	1.44	0.91
Almond seed oil (*Prunus arnygdalyus*)	Liquid	Pale yellow	1.46	0.91
Wild apricot (*Prunus armeniaca*)	Liquid	Pale yellow	1.46	0.91
Bhekal (*Prinsepia utilis*)	**Liquid**	**Yellow**	**1.36**	**0.84**

The chemical properties of oil are among the most important properties that determine the present condition of oil (Nzikouet al., 2009). Among these oils, yield of kernel is similar to the oil yield of almond oil (58.9%) and sunflower oil(52.1%) as reported by Bachheti et al., 2012, and higher than that of many conventional oil seed crops like cotton (15.0-24.0%), soybean (17.0-21.0%) reported by Pritchard,1991, Mustard seed oil (39.7%), ground nut seed oil (40%), linseed oil (37.1), coconut seed oil (39.0%) and wild apricot (44.3%). In the agricultural economy of India, oilseeds are important next only to food grains in terms of production and value. In view of the increasing demand of edible oil, government of India is promoting nonconventional source of edible oil which would give high per unit area production (Motilal, 1996). This high percentage oil yield of bhekal in this study show that the industrial processing of the oil for soap making and edible purposes would be viable.

The acid value of bhekal oil is found 3.78mg/KOH/g is nearest to linseed oil, sunflower seed oil, ground nut oil, coconut oil, almond and apricot seed oil which is already in use for edible purposes and this fall within the recommended codex of 0.6 and 10 for virgin and non-virgin edible fats and oils respectively (Dawodu, 2009). Aremu et al., 2015 and Akubor et al., 2008, also reported that low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This essence suggests that the bhekal oil is suitable for edible purposes and also in the

manufacture of paints and varnishes (table-6). The iodine value of bhekal oil was found 142 g/100g; indicates the degree of saturation of oil. Aremu et al. (2006a) reported that the lower the iodine value, the lesser the number of unsaturated bonds; thus, the lower the susceptibility of such oil to oxidative rancidity. The saponification value of bhekal oil is 220 mg/KOH/g and it falls within the range of values obtained for some vegetable oil 188 - 235mgKOH/g (Aremu et al., 2006).(Motilal, 1996). This high percentage oil yield of bhekal in this study show that the industrial processing of the oil for soap making and edible purposes would be viable. The acid value of bhekal oil is found 3.78mg/KOH/g is nearest to linseed oil, sunflower seed oil, ground nut oil, coconut oil, almond and apricot seed oil which is already in use for edible purposes and this fall within the recommended codex of 0.6 and 10 for virgin and non-virgin edible fats and oils respectively (Dawodu, 2009). Aremu et al., 2015 and Akubor et al., 2008, also reported that low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This essence suggests that the bhekal oil is suitable for edible purposes and also in the manufacture of paints and varnishes (table-6). The iodine value of bhekal oil was found 142 g/100g; indicates the degree of saturation of oil. Aremu et al. (2006a) reported that the lower the iodine value, the lesser the number of unsaturated bonds; thus, the lower the susceptibility of such oil to oxidative rancidity. The saponification value of bhekal oil is 220 mg/KOH/g and it falls within the range of values obtained for some vegetable oil 188 - 235mgKOH/g (Aremu et al., 2006).

Table-6 Chemical properties of some common edible oils and bhekal oil

Species	**AV** [a]	**IV** [b]	**UM** [c]	**SV** [d]	**FFA** [e]	**TS** [f]	**TU** [g]
Soya been oil (*Sojamax*)	1.08	132.0	0.33	192	0.56	14.39	85.66
Mustard seed oil (*Brassica compestris*)	1.21	108.0	0.35	174	0.59	8.79	91.21
Ground nut oil (*Arachis hypogoes*)	3.98	88.0	0.62	193	1.74	13.11	81.89
Cotton seed oil (*Gossium*)	0.26	108.0	0.86	195	0.10	22.56	77.4
Linseed oil (*Linum usitatissum*)	3.42	174.0	1.09	189	1.61	10.54	89.46
Sunflower Seed oil (*Helianthus annus*)	3.89	128.0	0.96	188	1.68	9.63	90.37
Coconut oil (*Cocos nucifera*)	3.62	8.4	0.43	261	1.79	92.68	8.92
Almond seed oil (*Prunus arnygdalys*)	3.42	96.0	0.49	191	1.72	9.0	86.0
Wild apricot seed (*Prunus armenica L*.)	4.05	102.0	0.71	190	2.01	7.17	93.13
Bhekal seed oil (*Prinsepia utilis*)	**3.78**	**142**	**0.02**	**222**	**13.90**	**21.80**	**34.60**

a: Acid value, b: Iodine value, c: Unsaponifiable matter (%w/w), d: Saponifiable matter,
e: Free fatty acid, f: Total saturated %, g: Total unsaturated (%).

Table-7 Mineral content (mg/100g) of common oils and bhekal oil

Species	P	Zn	Ca	Mg	K	Na	Fe	Cu
Soya been oil (*Sojamax*)	690.2	3.41	242.6	178.9	0.009	0.011	11.1	1.14
Mustard seed oil (*Brassica compestris*)	694.3	4.86	492.1	0.034	0.019	0.007	8.11	0.84
Ground nut oil (*Arachis hypogoes*)	349	3.91	86.4	0.048	0.007	0.011	2.62	0.92
Cotton seed oil (*Gossium*)	256	1.25	135.6	1.23	----	---	2.89	0.86
Linseed oil (*Linum usitatissum*)	374	3.68	168.9	0.021	0.09	0.026	2.76	1.92
Sunflower Seed oil (*Helianthus annus*)	671.0	5.31	280.0	0.096	0.042	0.011	4.84	1.61
Coconut oil (*Cocos nucifera*)	208	4.96	402.8	0.084	0.86	0.41	7.89	1.33
Almond seed oil (*Prunus arnygdalys*)	484	3.61	239.2	374.6	0.019	0.054	4.26	0.98
Wild apricot seed (*Prunus armenica*)	472	3.79	330.0	370.0	0.017	0.34	3.6	1.56
Bhekal seed oil (*Prinsepia utilis*)	**428**	**3.19**	**312**	**3.21**	**1.02**	**0.48**	**3.90**	**1.32**

Bhekal oil have nearly same ratio of oleic acid and just double of fatty acid as compared to coconut oil and significantly lesser than that of other taken CUOs e.g. mustard oil, cotton seed oil, soybean oil, linseed oil, almond oil, except coconut oil which contains high amount of saturated fatty acids. The results of comparison, in between different CUOs and wild bhekal seed oil, clearly indicate that the values of chemical properties like acid value, iodine value, saponifiable matter, are highly comparable with sunflower oil and almond seed oil (Prunus arnygdalys). Hence with these chemical values, the wild bhekal seed oil may also be suggested as a good alternative for sunflower oil and almond seed oil in industries and food purposes.

Conclusion

The present study envisages that bhekal seed oil from the Chakrata forest division have almost same Physico-chemical properties as in other conventional oils. Bhekal seed have higher yield of oil over 50%, which is comparable to the oil yield of some commercial seed oils such as soyabean oil, mustard seed oil, ground nut oil, cotton seed oil, coconut oil, wild apricot oil But lower than almond seed oil and sunflower oil. Many of the physico-chemical properties of the seed oil studied have close similarity with other commercial seed oils. The results obtained from this study could be used as baseline data to develop bhekal oil (*P. utilis*) for both domestic and industrial purposes and also for promotion and cultivation of this shrub with a sustainable manner in the Garhwal (India) region for large scale production of oil.

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In vitro Antimicrobial and Antioxidant Activity of Immunity Booster Tea Formulation,

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DOI 10.51129/ujpah-2020-29-2(2)

Abstract-Currently, there is much growing interest in the use of medicinal plants as modulators of the complex immune system. Through a number of vast researches conducted in the area, it is being explored that many of the phytochemicals in the form of alkaloids,tannins, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties. Keeping in mind, the tremendous potential of the medicinal plants and their derived drugs, this study is undertaken and an immunobooster tea formulation (IMBF-01-20) was designed and *in vitro* antimicrobial and antioxidant activity was tested. The results showed strong antimicrobial activity of formulation(IMBF-01-20) against *Staph.aureus* with diameter of zone of inhibition of 32 mm in methanol extract followed by 20 mm in *E.coli.* While hexane extract also showing good inhibition of test organisms followed by Ethanol extract.Considering the growing demand of natural antioxidant, IMBF-01-20 was tested for their antioxidant activity using DPPH radical scavenging assay.Antioxidant activity (93.4%) was observed in immunobooster tea formulation and revealed it as a natural antioxidant.

Key words: Immunomodulators, Medicinal Plants, Immunobooster tea, Phytochemicals

Introduction

A broad range of health-care practices is required to exploit the beneficial effects of Ayurveda which is the most ancient 5000 years old system of medicines. Being the essence of Ayurvedic medicines, Indian medicinal plants manifest miraculous effects in curing a vast range of diseases and disorders among humans and can be better called as "elixirs of life." Currently, there is much growing interest in the use of these medicinal plants as modulators of the complex immune system. Through a number of vast researches conducted in the area, it is being explored that many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties.**(Preeti Sharma, et. al, 2017)**

Keeping in mind, the tremendous potential of the medicinal plants and their derived drugs, the present work done with a purpose to globally popularize the Indian herbal medicines as immunomodulators.

Concerted efforts have been made to explore the various biological and specific pharmacological activities and their active compounds all over the world. The antibacterial and antifungal activities of Indian medicinal plants are widely known against a variety of pathogenic and opportunistic microorganisms (Aqil and Ahmad, 2007). However, targeted screening with improved strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy.

It is expected that plant extract showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.,* 1998). The selection of Herbal ingredients were based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et. al.,* 1999).

In the recent years, based on leads from Ayurvedic system and other traditional medicine system, several antiviral agents have been isolated from plants. These agents include a variety of polyphenols, flavonoids, saponins, glucosides, and alkaloids. Some traditional Indian spices and herbs are taken as ingredeints in immunity booster formulation IMBF-01-20 with an aim to prepare the body to fight infection.

The search for natural antioxidants has generated interest among scientific community to reinvestigate

Indian Herbs as safe and promising antioxidant agent (Wannes et al., 2010; Tongpoothorn et al., 2012; Kapoor et al., 2014).

The current research in the area to develop plant-derived natural products as potent and safer leads to act as Generation of herbal medicine as multiple-component agent expected to modulate the complex immune process in such a way so as to prevent the infection rather than treatment and cure of the disease. With all these aspects keeping in mind, the present work focuses on an immunobooster tea formulation with its *in vitro* antimicrobial and antioxidant activity.

Material and methods

Plants material

The authentic plant/Herbs were obtained from the Himalaya Drug Company, Dehradun. The identification of the samples was further confirmed by the plant taxonomist, Dr. Maya Ram Uniyal Former Jaribooti Expert Govt.of Uttarakhand, Dehradun. The voucher specimen has been deposited in the Herbal Museum The Himalaya Drug Company Dehradun Uttarakhand.

Bacterial strains used in the screening programme

The Standard strains were obtained from HiMEDIA**,** Mumbai include the ATCC strains of *Staphylococcus aureus* (MRSA), *and* strains of *E. coli.*

Culture Media and Inoculum preparation

Nutrient broth/ Agar and MullerHinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 ^{0}C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^{5} 10^{6}CFU/ml.

Preparation of plant extracts and its fractionation

The extract of IMBF-01-20 was prepared as described earlier (Ahmad and Beg 2001) with a little modification. 800 gram of dry, powder was soaked in 1.5 liter each of Methanol, Hexane and 70% ethanol, for 36 days and stirred after every 10 hr using a sterilised glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates were concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO(Dimethylsulpoxide), to have a stock solution of 100 mg/ml concentration.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Beg, 2001) was used. 0.1 ml of diluted inoculum (10^{5} CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

DPPH radical scavenging assay

Free radical scavenging activity of Immunobooster tea formulation against stable 1, 1,diphenyl-2-picrylhydrazyl (DPPH) was determined spectrophotometrically by slightly modified method of Gyamfi et al. (1999) as described below. When DPPH reacts with an antioxidant, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm on a UV/visible light spectrophotometer (Spectronic 20 D+, Thermo Scientific, USA). Fifty µl of Immunobooster tea extract in DMSO, yielding different concentrations was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mMTris-HCl buffer (pH 7.4). DMSO (50 µl) was used as a vehicle control in the experiment. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured spectrophotometrically. Ascorbic acid and butylated hydroxytoluene were used as positive controls. Inhibition percent was calculated from the following equation:

% Inhibition = [Absorbance of control Absorbance of test sample / Absorbance of control] × 100

Results and Discussion

In the present study, Herbs were selected on the basis of their traditional uses in treatment of different diseases in India and worldwide. Only alcoholic extracts of Immunobooster tea formulation have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff,1998;Ahmad et al.,1998).

Antibacterial activity of Immunobooster tea against Gram positive bacteria (*S. aureus ATCC- 6538*) and Gram- negative bacteria (*E. Coli*, ATCC-8739) is presented in Table-1, Plate No-1-5. Activity of Methanolic extracts against Gram positive bacteria showed strong zone of inhibition of 32 mm followed by 20 mm against Gram negative bacteria(Plate-1&4). On the other hand Hexane extract showed 19mm and14mm against gram positive and gram negative respectively Followed by Ethanol extract. Most potential extract was methanol extract followed by Hexane and Ethanol as depicted in plate-1 to 5.

Antioxidant activity

The immunobooster tea formulation IMBF-01-20 under study was subjected to antioxidant scrutiny by standard methods namely DPPH free radical scavenging activity .The sample was ten times diluted in dimethyl sulpho-oxide and were tested at concentration ranging from 100-500 µg/ml. IMBF-01-20 demonstrated strong DPPH scavenging activity (>93.54% decolorization) at 500 µg/ml concentrationas shown in Table-2. The values were comparable to commercial standards ascorbic acid (94.9%) and BHT (92.8%).

The antioxidant potential of essential oils highlights their therapeutic potential both in traditional and modern phytomedicine. Since essential oils in herbs/culinary herbs and spices consist of terpenes and other different kind of phenolic compound, it would seem reasonable that their mode of action might be related to those of other phenolic compounds (Tassou et al., 2000) and contribute to their antioxidant activity.

Our findings are in agreements with reports of various other workers who have investigated for the similar antioxidant potential of essential oils and their active compounds from India as well as other parts of the world (Dorman et al., 2000; Manuel et al., 2010; Zahin et al.,2010; Vaibhavi et al., 2010). However, the activities slightly differ due to difference of minor phytoconstituents and the variability in the quantity of major active constituents which may arise due to different agroclimatic condition, plant varieties and extraction processes.

Various herbal medicines have been found to modulate various components of innate and acquired immune system. In fact, based on proper understanding of various immunomodulatory activities of herbal plants, plants derived the secondary metabolites in natural products can be the lead molecules for the future development of immunomodulators for therapeutic use. Various immunomodulators have been suggested in various allergic diseases including asthma, allergic rhinitis, and eosinophilic esophagitis on the basis of experiments performed on various animal models.

Conclusion

On the basis of preliminary investigation of the present study, it may be concluded that the tested immunobooster tea formulation have the potential for application in real food system and healthcare.

This preliminary investigation indicated that potential plants/herbs extracts showing broad spectrum antimicrobial activity could be further tested to determine the efficacy in-vivo against MDR bacteria. Active fractions of various plants may also be exploited in preparation of herbal formulation of improved efficacy and quality.

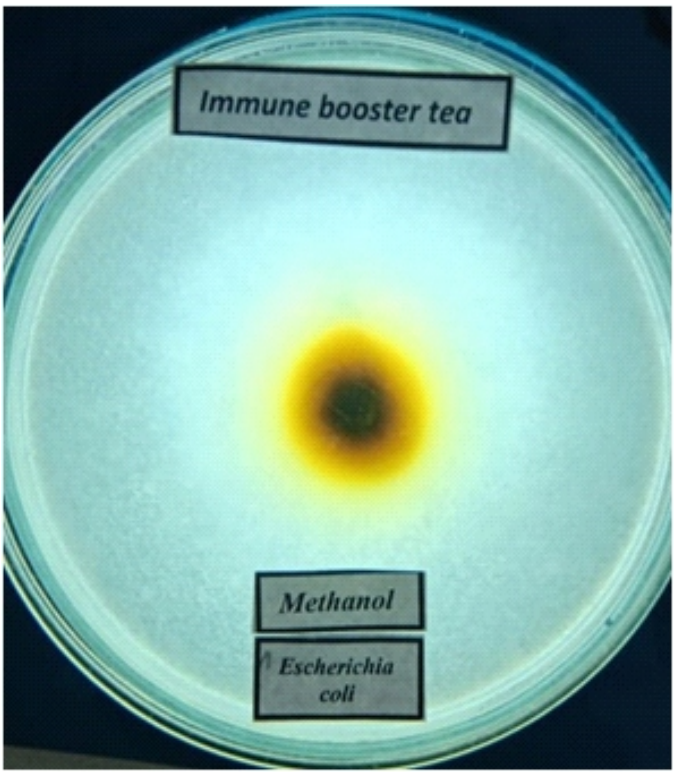
Many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties.

Table-1: Antimicrobial activity of Immunobooster tea formulation

S.No.	Extract	Diameter of zones of inhibition(mm)		
		Staph.aureus	*E.coli*	Ciprofloxacin (as positive control)
1.0.	Methanol	32	20	34 for *Staph aureus*and 33 for *E.coli*
2.0.	Hexane	19	14	
3.0.	Ethanol	17	15	

Table-2 Antioxidant activity of Immunobooster tea Formulation

S.No.	Test sample	Antioxidant activity in %(DPPH method)
1.0.	Immunobooster tea Sample	93.54%
2.0.	Ascorbic Acid	94.9%
3.0.	BHT	92.8%.



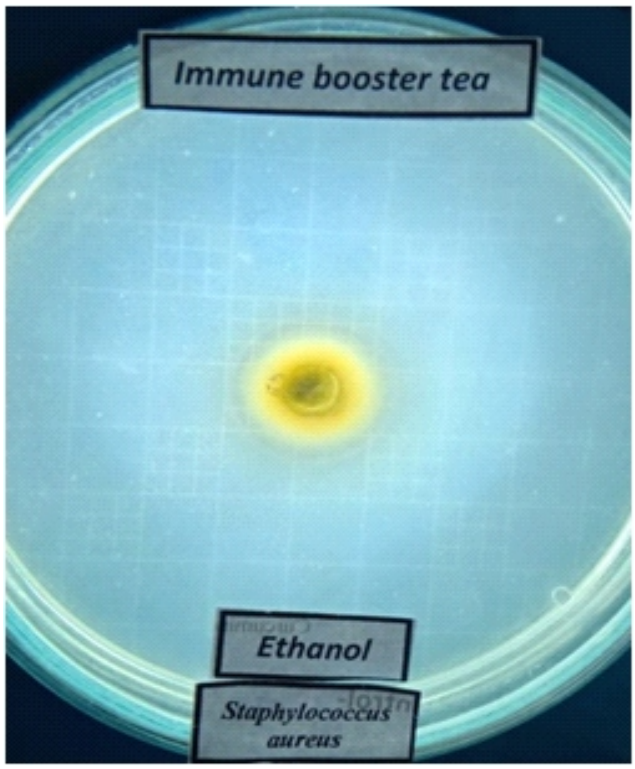
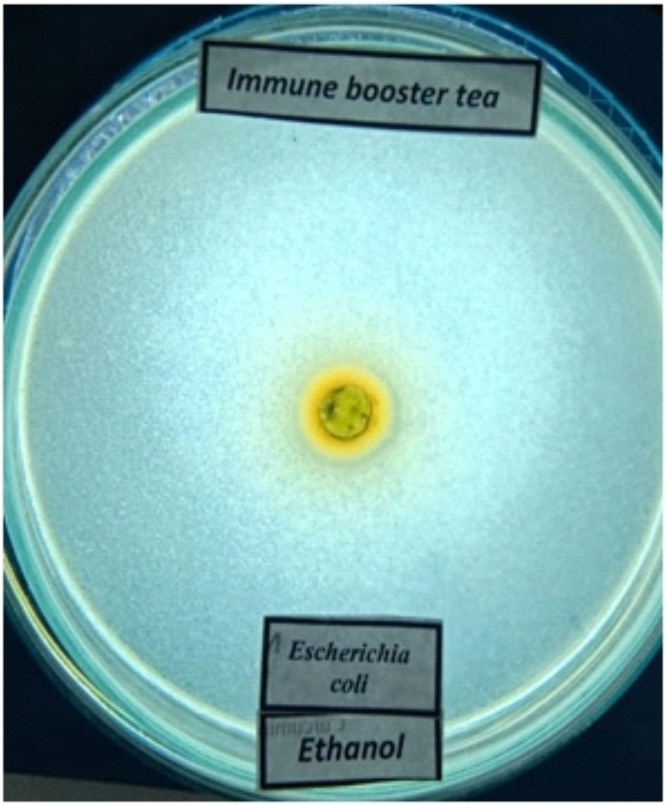


PLATE-1 Antimicrobial activity of methanol extract against EC
PLATE-2 Antimicrobial activity of ethanol extract against SA



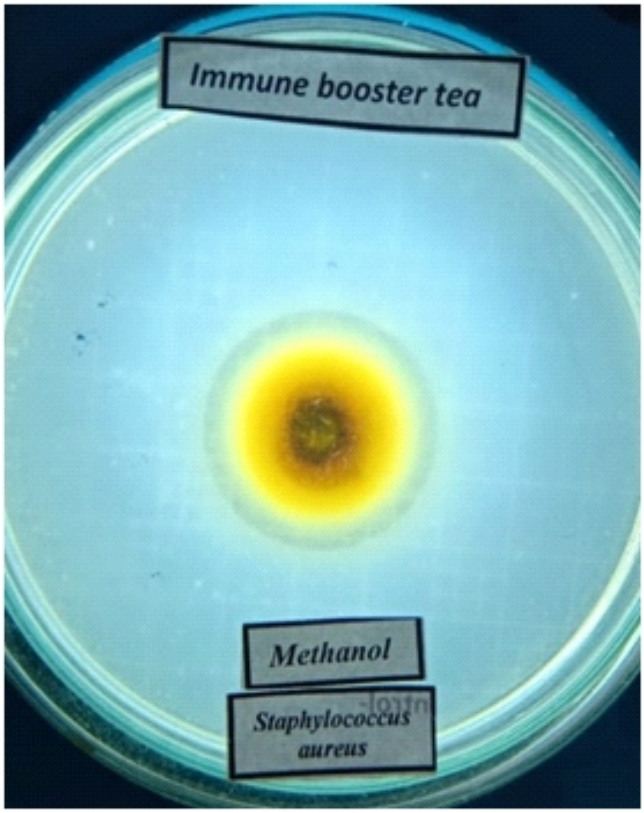


PLATE-3 Antimicrobial activity of ethanol extract against EC
PLATE-4 Antimicrobial activity of methanol extract against SA

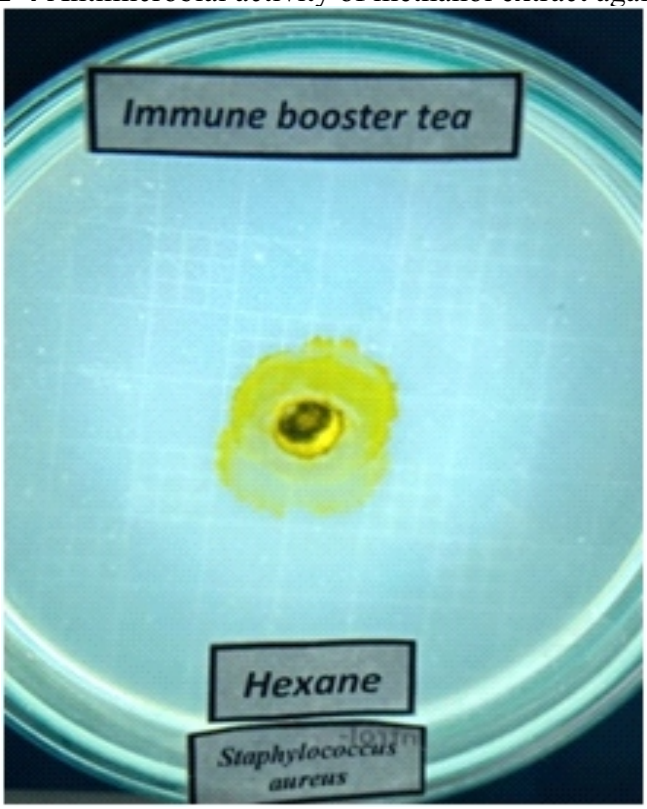


PLATE-5 Antimicrobial activity of Hexane extract against SA
Note: EC; *E. Coli* & SA; *Staph aureus*

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Assessment of *In Vitro* Sun Protection Factor of Plant Extracts by Ultraviolet Spectroscopy Method

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DOI 10.51129/ujpah-2020-29-2(3)

Abstract-Exposure to sunlight can trigger various biological responses ranging from sun-burn, erythema to skin cancer. Synthetic sunscreen formulations available in market pose variety of adverse effects. Therefore, formulation of the herbal sunscreen formulation and evaluation of its sun protection activity is an important aspect in the cosmetic industry. The purpose of present study was to evaluate the sun protection factor (SPF) of aqueous and methanolic extract of *Aloe barbadensis miller* and *Cocos nucifera* by ultraviolet (UV) spectroscopy method. Methanolic extract of Cocos nucifera showed the highest SPF value (3.2**)** amongst all extracts. The results indicated presence of active components responsible for ultraviolet absorption which may be extracted from these plant extracts and maybe used in sunscreens preparations for better protection against sun rays.

Key Words: UV radiations, SPF, *Aloe barbadensis miller* and *Cocos nucifera*

Introduction

The human body is constantly exposed to an array of chemical and physical exogenous pollutants (Perluigi et al. 2010). The harmful effects of solar radiations are caused predominantly by the ultraviolet (UV) region of the electromagnetic spectrum, which can be divided into three regions: UVA (400-320 nm) UVB (320-290nm) and UVC (290-200 nm) (Dutra et al. 2004). UVC radiations are filtered out by the atmosphere before reaching earth. UVB radiations are not completely filtered out by the ozone layer and are responsible for the damage due to sunburn and pyrimidine dimmers formation. UVA radiation reaches the deeper layers of the epidermis, dermis and provokes the premature ageing of the skin and is responsible for the generation of free radicals. UVB radiations are involved in 65% damage of all skin and are responsible for causing sunburn, cutaneous degeneration, photosensensitivity, phototoxicity, and ectinic elastisis (Hawk, 2001; Mbanga et al. 2014). The main destroying factors of UV radiations for skin are oxygenated molecules which are often call free radicals such as; superoxide anions (O2.-), hydroxyl radical (.OH), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. It is well documented that ultraviolet (UV) light indices immune suppression and oxidative stress, which play an important role in the induction of skin cancers (Mukhtar and Elmets, 1996). Previous reports have suggested that ultraviolet (UV) radiation is responsible for distinct mutations in keratinocytes that ultimately contribute to the development of the non-melanoma skin cancers, which include basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Thus it becomes necessary to protect the skin from such carcinogenic radiation. Sunscreens and sunblocks are chemicals that absorb or block UV rays and show a variety of immunosuppressive effects of sunlight. There are several agents available from both synthetic and natural sources with UV-filtering properties. Given their potential to produce considerable human local and systemic exposure, UV filters have to be safe (Nohynek *et al.,* 2010). Synthetic UV filters are known to have potential toxicity in humans and also showed ability to interfere only in selected pathways of multistage process of carcinogenesis (Chanchal and Swarnlata, 2009).To stimulate the skin, to repair and build itself naturally, we need find an arsenal of potent ingredients. In a quest to find effective topical photoprotective agents, plant-derived products have been researched because of their antioxidant activity mostly due to presence of phytochemical substances. Effective botanical antioxidant compounds are widely used in traditional medicine including tocopherols, flavonoids, phenolic acids, nitrogen containing compounds (indoles, alkaloids, amines, and amino acids), and monoterpenes. The sun protection factor of a sunscreen is a laboratory measure of the effectiveness of sunscreen, the higher the SPF, the more protection a sunscreen offers against UV-B (the ultraviolet radiation that causes sunburn) (Ramos-e-Silva and Carneiro, 2007). The SPF is the

amount of UV radiation required to cause sunburn on skin with the sunscreen on, relative to the amount required without the sunscreen (FDA, 2009). There is an immense need to explore the sunburn protective properties of herbal plants because of their proved medicinal properties due to rich source of phytoconstituents and oxidation inhibitors. Keeping the above factors in consideration, the present study was planned to evaluate SPF vaules and phytochemical properties of *Aloe barbadensis miller* and *Cocos nucifera*.

Material and Methods

Sample Preparation

The Coconut and *Aloe vera* samples were washed with distilled water twice and dried. The samples were then grinded separately in a mixer grinder and 20 gm from each were taken separately in a beaker. This powder was weighed and loaded to the Soxhlet apparatus and 200 ml of methanol/water was used as solvent for preparing the alcoholic and aqueous extract respectively. The extraction process was carried out overnight and the extract was then filtered .The filtered extract was suitably diluted with methanol/water or dried and kept at 4˚C for measuring SPF and phytochemical anaylsis.

Phytochemical Analysis Of Plant Extract

Flavonoid Test: 5ml of diluted ammonia solution was added to aqueous filtrate of the plant extract followed by the addition of concentrated H_2SO_4. Formation of yellow color indicates the presence of flavonoids.

Saponins Test: One ml of plant extract was diluted with 20 ml distilled water and the tube was shaken. Formation of foam indicates the presence of saponins.

Alkaloids Test: **To one** ml of plant extract, 3ml of ammonium solution was added and incubated at 37 ˚C for few minutes. The tubes were then placed in water bath and then mayer's reagent was added. Formation of cream color precipitation showed the presence of alkaloids.

Terpenoids Test: 2ml of chloroform was added to 5ml of plant extract. Conc. H_2SO_4 (3ml) was then carefully added to form a layer. Reddish brown coloration of the interface indicated the presence of terpenoids.

Carbohydrate Test: 1ml of Fehling's A and Fehling's B were heated in a boiling water bath for 5-10 min with the plant extract. Appearance of reddish orange precipitate shows the carbohydrate presence.

Tanins Test: About 2ml of the plant extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution (5%w/v) were added. The formation of a green precipitate was an indication for the presence of tannins.

Phenol Test: **Plant e**xtracts were treated with 3-4 drops of $FeCl_3$ solution. Formation of bluish black colour indicates the presence of phenols.

Steroid Test: A red colour produced in the lower chloroform layer on addition of 2 ml plant extract to 2 ml of chloroform and 2 ml conc. H_2S0_4 indicates the presence of steroids.

Determination of SPF

100 mg of aqueous and methanolic extract were dissolved in 100ml of distilled water. From this 2 ml and 4ml of the extract was withdrawn and diluted to 10 ml with distilled water so as to prepare extract with the final concentration of 200µg/ml and 400µg/ml. Thereafter, the absorbance of these extracts was taken by spectrophotometer from wavelength ranging from 290 to 320 at 5nm. SPF for aqueous and methanolic extract was calculated by the formula given by Mansur equation and by utilizing values given by Sayre (Kaur and Saraf, 2010). SPF was calculated three times and then mean value was taken in consideration.

In vitro SPF can be calculated by following equation**:**

$$\mathbf{SPF = CF \times \sum EE \times I \times Abs}$$

Where;

(I) - the solar irradiance spectrum,

EE (I) - the erythemal action spectrum,

Abs- absorbance of sunscreen product,

CF-correction factor (=10)

The value of EE x I are constant and predetermine as shown in table-1

Table-1 Values of EE×I used in the calculation of SPF

Wavelength (nm)	EE*I (Normalised)
290nm	0.0150
295nm	0.0817
300 nm	0.2874
305nm	0.3278
310nm	1.864
315nm	0.0839
320nm	0.0180
TOTAL	1

Results and Discussion

3.1 Phytochemical Analysis Of Plant Extracts

Phytochemical examination revealed the presence of constituents such as carbohydrates, alkaloids, glycosides, saponins, tannins, flavinoids, phenol and terpenoids (Table -2).

Table-2 Phytochemical Analysis

Constituents	Aloe vera (*Aloe barbadensis Miller*)	Coconut (*Cocos nucifera)*
Carbohydrates	+	+
Saponins	+	+
Alkaloids		+
Tarpinoids	-	+
Flavonoids	+	+
Phenols	-	+
Tannin	+	+
Steroids	-	+

Raphael (2012) conducted a study on phytochemical analysis of Aloe vera. Their study revealed the presence of tannins, flavonoids, terpenoids, carbohydrates, and alkaloids in *A. vera* plant whereas saponins, glycosides phlobatannins, antiquinones carbohydrates, and steroids were absent. Odenigbo and Otisi (2011) conducted study on phytochemical analysis of Coconut oil and reported the presence of alkaloid, resins, glycosides, terpenoids and tannins.

Determination of SPF

The sun protection factor or SPF is a measure of the fraction of damage-producing UV rays that reach the skin. It also gives an idea of how much time you can stay in sun without any protection (sunscreen) and without any damage to the skin. In order to protect against UV radiations, the formulation should have good SPF number and also the formulation should have wide range of absorbance between 290-400 nm ranges. In the present research work aqueous and methanolic extract of *Aloe barbadensis miller* and *Cocos nucifera* were subjected for SPF evaluation by UV spectroscopic method. SPF value for sunscreen above 2 is considered as having good sunscreen activity. The calculated values of SPF of aqueous and methanoic extract of *Aloe barbadensis miller and Cocos nucifera* are presented in the table-3-6 *SPF value of* aqueous extract of *Cocos*

nucifera was 0.647 and 1.454 at concentration of 200µg/ml and 400µg/ml respectively. Methanolic extract of Coconut have SPF value 1.305 and 3.207 at a concentration of 200µg/ml and 400µg/ml respectively. It was found that aqueous extract of have *Aloe barbadensis miller* SPF value of 0.14802 and 0.082276 at concentration of 200µg/ml and 400µg/ml respectively. Methanolic extract of *Aloe barbadensis miller* have SPF value 0.60412 and 1.96168 at a concentration of 200µg/ml and 400µg/ml respectively.

Table-3 Absorbance and SPF value of methanolic extract of *Cocos nucifera*

S.No	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.845	0.012675	2.022	0.03033
2	**295**	0.817	0.789	0.644613	1.923	1.571091
3	**300**	0.2874	0.748	0.2149752	1.845	0.530253
4	**305**	0.3278	0.718	0.2353604	1.783	0.5844674
5	**310**	0.1864	0.695	0.129548	1.726	0.3217264
6	**315**	0.0837	0.682	0.0570834	1.678	0.1404486
7	**320**	0.018	0.642	0.011556	1.63	0.02934
	Total			**1.305811**		**3.2076564**

Table-4 Absorbance and SPF value of aqueous extract of *Cocos nucifera*

S.No	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.443	0.006645	0.991	0.014865
2	**295**	0.817	0.4	0.3268	0.894	0.730398
3	**300**	0.2874	0.37	0.106338	0.83	0.238542
4	**305**	0.3278	0.348	0.1140744	0.785	0.257323
5	**310**	0.1864	0.332	0.0618848	0.752	0.1401728
6	**315**	0.0837	0.319	0.0267003	0.724	0.0605988
7	**320**	0.018	0.306	0.005508	0.699	0.012582
	Total			**0.6479505**		**1.4544816**

Table-5 Absorbance and SPF value of aqueous extract of *Aloe barbadensis miller*

S.No.	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.111	0.001665	0.059	0.000885
2	**295**	0.817	0.101	0.082517	0.053	0.043301
3	**300**	0.2874	0.091	0.0261534	0.047	0.0135078
4	**305**	0.3278	0.083	0.0272074	0.043	0.0140954
5	**310**	0.1864	0.038	0.0070832	0.038	0.0070832
6	**315**	0.0837	0.034	0.0028458	0.034	0.0028458
7	**320**	0.018	0.031	0.000558	0.031	0.000558
	Total			**0.1480298**		**0.0822762**

Table-6 Absorbance and SPF value of methanolic extract of *Aloe barbadensis miller*

S.No.	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.152	0.00228	1.192	0.01788
2	**295**	0.817	0.140	0.11438	1.162	0.94935
3	**300**	0.2874	0.129	0.03672	1.146	0.32936
4	**305**	0.3278	0.101	0.03310	1.112	0.36451
5	**310**	0.1864	0.091	0.01696	1.106	0.21361
6	**315**	0.0837	0.083	0.06947	0.910	0.07616
7	**320**	0.018	0.041	0.00073	0.601	0.01081
	Total			**0.60412**		**1.96168**

The different SPF values of *Aloe barbadensis* miller and *Cocos nucifera* indicates that aqueous and methanolic extract of them found near the range of good sunscreen activity. Methanolic extract of *Cocos nucifera* showed the highest SPF value amongst all extracts. Thus it can be proposed that these plant extract can absorb the ultraviolet radiation since they possess good sun protection activity against ultraviolet radiations.

Malsawmtluagi et al. (2013) conducted a similar study on some aqueous extract of vegetables to determine the ultraviolet (UV) absorption properties determining the sun protection factor (SPF) number. It was observed that all of the tested herbals showed some UV protection capabilities with aqueous Coconut extract showing the highest SPF number of 7.38 while watermelon showed the lowest SPF number of 0.97. Suva (2014) conducted a study to evaluate the sun protection factor (SPF) of methanolic extract of flowers and leaves of *Azadirachta indica* by ultraviolet (UV) spectroscopy method. It was reported that methanolic extract of leaves of *Azadirachta indica* have SPF value about 1.47658 and methanolic extract of flowers of *Azadirachta indica* have SPF value about 1.3565.

Conclusion

The SPF values of the aqueous extracts of some *Aloe barbadensis miller and Cocos nucifera* were evaluated. It was found these extracts possess UV protection capabilities and active components responsible for ultraviolet absorption which maybe isolated from these plant extracts. Along with their many beneficial effects and safety, these botanicals could thus become good, cheap and easily available formulation ingredients in sunscreen products.

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Antioxidant Effect of *Stevia Rebaudiana* on Humanerythrocytes

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DOI 10.51129/ujpah-2020-29-2(4)

Abstract-The changing lifestyle and environment conditions have predisposed common man towards numerous diseases. Today most of the diseases are said to be caused by synthetic chemicals, toxic heavy metals, and the stress of modern living. It is also true that oxygen is essential for sustaining life but it is also dangerous to our existence. Oxygen is being viewed as playing a lead role in the generation of reactive intermediates, thereby causing cellular damage. Our body has the mechanism to handle free radicals and prevent its damaging effect, which involves the use of antioxidants as glutathione and antioxidant enzymes as superoxide dismutase, catalase, glutathione peroxidase glutathione reductase, glutathione-S-transferase to counter these free radicals. When the redox status of the body is overwhelmed by these radical species, this may result in variety of chronic diseases and even premature senility. The administration of natural antioxidant as food constitutes or therapeutic agents is looked-for to neutralize these reactive oxygen species and prevent or delay diseased condition caused by these reactive species. Most exogenous antioxidants come from raw vegetable fruits, spices, herbs and various medicinal plants.Natural antioxidants are always appreciated over synthetic ones because they lack toxic side effects.

The present study deals with the effects of *Stevia rebaudiana* leaf extract on the status antioxidant of RBC as evident by an *in vitro* dose-dependent decrease in the activity of erythrocytes superoxide dismutase and catalase as compared to the normal control whereas at much higher concentration of *stevia* leaf extract (100µg/ml) started to show a reversing trend of its protective action.
Keywords: *Stevia*, Antioxidants, Free radicals, ROS, Superoxide Dismutase (SOD), Catalase**.**

Introduction

The recent growth in the understanding of free radicals in biology has produced a medical revolution that potentials a new age of health and disease management (Aruoma, 2003). It is ironic that oxygen, which is essential for life under certain circumstances also can show damaging effects on the human body (Bagchi and Puri,1988). Most of the potentially damaging effects of oxygen are due to the formation of a number of chemically active compounds, known as reactive oxygen species (ROS), which have a tendency to contribute oxygen to other substances. Antioxidants which normally neutralize these reactive oxygen species and resulting into free radicals have been commonly used in modern clinical system to explain the mechanisms for causing disease (Aruoma, 1994).

During metabolism and exposure to external environment, a large amount of free radicals are produced in human body that can attack various biological macromolecules such as proteins, fatty acids and nucleic acids, causing oxidative damage to cells, tissues or even result in mutation of genes(*Young and Woodside, 2001)*.These radicals at high concentration cause oxidative stress, this destroys internal redox balance and can even result in a variety of chronic diseases(Gupta et al., 2014). Researches show that many diseases including cancers, arteriosclerosis, diabetes, cataract, cardiovascular diseases, Parkinson's disease, Alzheimer's disease and arthritis, correlate with cellular redox imbalance and free radical's generations(Labat-Robert and Robert, 2014). To scavenge excessive free radicals and maintain homeostasis, at the same time to prevent or delay diseases conditions, consumption of antioxidants become necessary.

Since, synthetic antioxidants to some extent have toxic effects, the uptake of natural antioxidants from natural sources becoming the first choice (*Papas, 1990*).Natural antioxidants not only play an important role in the prevention and treatment of disease conditions but also can avoid the adverse reactions caused by the ever increasing stress condition to human health (Liguori, et al., 2018)

Though oxidation reactions are essential for life, they can also be injurious. Inadequate levels of antioxidants or functioning of antioxidant enzymes, can cause oxidative stress and can result in damage to cellular components, including proteins, lipids and nucleic acids (Blokhina, et al., 2003). Under normal situations, the endogenous antioxidants in cells control the damage caused due to reactive oxygen species. However, exposure to adverse factors, abiotic or biotic causes excess generation of reactive oxygen species that cannot be efficiently controlled by the body defense system. This leads to oxidative stress where there is an imbalance between reactive species production and its elimination by antioxidants defense system. (De Gara, 2010). Antioxidants are reducing agents that include thiols, ascorbic acid, or polyphenols molecules that prevents oxidation of other molecules by getting oxidized themselves. Plants and animals maintain a systems of antioxidants such as glutathione, vitamin C, vitamin A and vitamin E as well as oxidant neutralizing enzymes such as catalase, superoxide dismutase and various peroxidases. For delay and prevention of a variety of diseases, antioxidants are widely used as dietary supplements and have been shown to be used as adjuvant in number of diseased conditions as cancer, coronary heart disease and even altitude sickness (Aune, 2019). Aging is another health threat related to stress, that affects whole populations and also can lead to premature mortality. The deleterious effects of free radicals on proteins, nucleic acids, and fats as well as enhanced glycosylation of proteins and DNA are widespread during aging processes(Maynard et al., 2015).Most exogenous antioxidant comes from vegetable, fruits, spices and herbs. They are important in food as they contain antioxidant molecules such as Alpha-tocopherol, vitamin A, vitamin B, Vitamin C and glutathione and can be called as natural antioxidants. Synthetic antioxidants like dimethyl sulphoxide, butylated hydroxytoluene and butylated hydroxyanisole, etc can also be used. But natural antioxidants are always preferred over synthetic ones, because antioxidants derived from natural sources show less toxic side effects and work by several mechanisms (Lourenço,et al., 2019). Therefore, in search of an effective antioxidant from natural source we studied the antioxidant property of *Stevia rebaudiana* leaves extract on human erythrocytes. Efforts were made to see its effect on the anti-oxidative stress related enzymes, superoxide dismutase (SOD) and catalase.

Material and Methods

Preparation of extract: Fresh leaves of *Stevia* plant were collected and dried under shade at room temperature. Dry powder of leaves was used for the extract preparation. Aqueouse thanol extract of *Stevia* leaves was prepared using the soxhlet apparatus following the methods of Da Porto et al. (Da Porto et al.,2016). In this process10 g grounded *Stevia* leaves were kept in extraction thimbles of the Soxhlet apparatus. Extraction was done for 12 hours at a maximum temperature of 70^0 C. After extraction, the residual solvent was removed at 50^0 C under reduced pressure using a vacuumdesiccator.

Isolation of erythrocytes - The blood was centrifuged at 4°C for 10 minutes at 3500 RPM to remove plasma and buffy coat, care was taken to eliminate leukocyte contamination. The isolated erythrocytes were washed with Krebs-Ringer phosphate buffer (KRPG buffer) (glucose 5mM, pH 7.4) in order to obtain completely washed packed erythrocytes, free of leukocyte contamination.

Treatment of isolated erythrocytes with extract - The packed erythrocytes were suspended in 4 volume of Krebs-Ringer phosphate buffer (KRPG buffer). Suspended erythrocytes were incubated with different concentration of *Stevia* leaves extract for 1 hour at 37°C. After incubation erythrocytes were washed with Krebs-Ringer phosphate buffer

(KRPG buffer) at 3500 RPM for 2-3 times and packed erythrocytes were obtained.

Preparation of Lysate -0.2 ml of packed RBC's was suspended in 3ml of Krebs-Ringer phosphate buffer (KRPG buffer, pH 7.4) and used for SOD whereas for catalase the above lysate was diluted 10 times with distilled water.

Estimation of Superoxide Dismutase (SOD):SOD was assayed according to the method Misra and Fridovich (1977) based on the ability to inhibit auto-oxidation of epinephrine to adrenochrome. The auto-oxidation of epinephrine in the solution at alkaline pH values, produce oxygen free radical, once formed precipitate in the oxidation of further molecules of epinephrine in a chain reaction to give rise to adrenochrome, which exhibits absorption maxima at 480 nm. Since SOD has the ability to inhibit this auto-oxidation at pH 10.2, thus inhibition can be measured spectrophotometrically at 480nm a per unit time of enzyme activity is equal to amount required to inhibit 50% of the above reaction in one min / mg protein.

To 0.25ml of lysate, 0.5ml carbonic buffer, 0.4ml EDTA (0.1mM), 0.25ml D.W. followed by addition of 0.1ml of epinephrine (0.3mM) just before taking the OD. Initial absorbance was noted at zero minute and readings were taken at every 30 seconds interval for 3 minutes at 480 nm using spectrophotometer against the blank. Protein content was estimated by Lowry's method.

SOD activity (unit/mg of protein)= ΔA_{480}/min x100xVolume of reaction mixture/mg protein.

Estimation of Catalase (CAT): Catalase activity in lysate was performed following the procedure of Beers and Sizer (1952). Catalase exerts a dual function because it catalyzes the following reaction.

$2H_2O_2 \longrightarrow 2H_2O+O_2$

$ROOH+AH_2 \longrightarrow H_2O+ROH+A$

In the UV range H_2O_2 shows a continued increase in absorbance with decreased wavelength. The decomposition of H_2O_2 can be followed directly by decrease in absorbance at 240nm. The difference in absorbance of H_2O_2 at 240nm per unit time is measure of catalase activity. To 1 ml of lysate, 1ml of phosphate buffer (50mM, pH- 7.4) followed by 1 ml of H_2O_2(30mM) just before taking the OD at 240 nm. The initial absorbance was taken at zero min followed by absorbance at every 30 second interval for 3 minutes at 480 nm. Protein concentration was estimated by Lowry's method.

Catalase (unit/mg of protein) = ΔA_{240} / minx Volume of reaction mixture x 100/ mg of protein

Results and discussion

Effect of *Stevia rebaudiana* leaf extract on SOD activity

The effect of SOD activity on different concentrations (0.01-100µg/ml) of *Stevia rebaudiana* leaves extract is shown in Figure-1. *Stevia rebaudiana* showed a significant decrease in SOD activity as compared to normal control up to a concentration of 10µg/ml of extract. *In vitro* treatment of RBC with *Stevia rebaudiana* leaves extract resulted in a dose dependent decrease in SOD activity with the increasing concentration of extract (0.01 - 10µg/ml).

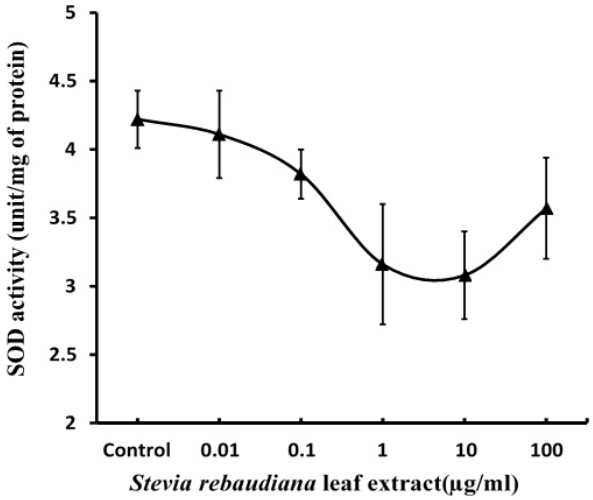

Figure -1

Figure-1 *In-vitro* effect of *Stevia rebaudiana* leaves extract on SOD activity of erythrocytes. Each value is the mean of at least 3-4 independent experiment. Values are expressed as mean ±S.D.

Our results further showed that treatment of erythrocytes with higher concentration of *Stevia*

rebaudiana leaves extract the effect of *Stevia rebaudiana* leaf extract on SOD activity started to reverse and became significantly increase at concentration of 100µg/ml, as compared to the maximum effect shown at 10 µg/ml extract.

Effect of *Stevia Rebaudiana* Leaf extract on Catalase activity

The effect of different concentrations (0.001-10µg/ml) of *Stevia rebaudiana* leaf extract on Catalase activity is shown in figure-2. *Stevia rebaudiana* leaf extract (0.01-.100µg/ml) showed a significant dose dependent decrease in Catalase activity as compared to normal control. Maximum effect was observed at a concentration of 1 µg/ ml. The effect of *Stevia rebaudiana* leaf extract at a concentration of 10µg/ml) started to decrease and catalase activity became significantly increased as compared to the maximum effect at 100 µg/ ml. Our results showed that erythrocyte catalase activity decreased with increasing concentration of *Stevia rebaudiana* extract up to a concentration of 10 µg/ml but further increase in *Stevia rebaudiana* extract at concentration **(**100µg/ ml.**)** resulted in increase in Catalase activity as compared to the maximum decrease at 1µg/ml.

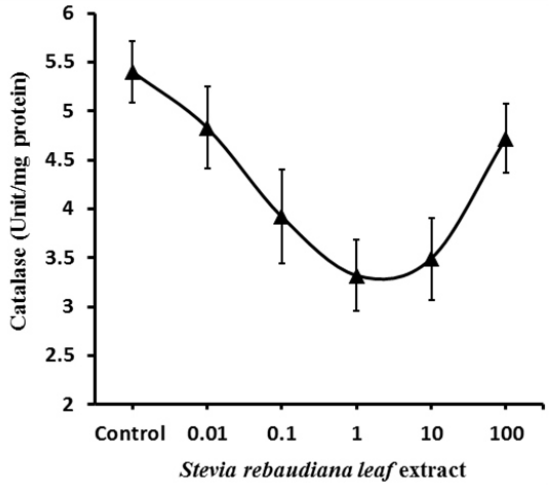

Figure-2

Figure-2 *In vitro* effect of *Stevia rebaudiana* leaves extract on erythrocytes catalase activity. Each value is the mean of at least 3-4 independent experiment. Values are expressed as mean± S.D.

ROS and other free radicals are nowadays being thought to be are involved in many disease conditions. ROS are produced in cells under normal physiological conditions and if left uncontrolled leads to formation of lipid peroxides and by subsequent chain reaction more free radicals are produced. Antioxidants help in eliminating these free radicals. Antioxidants minimize the action of ROS, compounds which act as antioxidants can trap the free radicals directly or scavenge them through a series of reaction coupled with anti-oxidative enzymes (Pizzino,*et. Al.*,2017).

We investigated the effect of Stevia leaf aqueous ethanolic extract on free radical scavenging enzymes Superoxide Dismutase and Catalase, the key component of the antioxidant defense mechanism. These scavenging enzymes play a significant role in removing of the ROS from the tissues. Superoxide Dismutase and Catalase provide a defense against the potential damaging action of superoxide and hydrogen peroxide. High Superoxide Dismutase activity in conjugation with catalase activity leads to increased level of hydrogen peroxide and hydrogen peroxide derived related species such as hydroxyl radical. Study proposes that the imbalance of Superoxide Dismutase and Catalase activity may play a role in initiating and propagating oxidative damage.(Maciejczyk,*et.al.*, 2019).Superoxide dismutase and Catalase provide a defense against the potential damaging action of superoxide and hydrogen peroxide.

Our results show a dose dependent protection of Superoxide dismutase and catalase activity upto a concentration of 10µg/ml extract. The decreased activity of CAT and SOD in our study could probably be associated with elevated exogenous antioxidant level, due to neutralization of peroxidant and free radical scavenging property of *antioxidant spresent in plants*(Prakash,*et al.*, 2007). The decreased activity of Catalase and Superoxide Dismutase observed in our study could probably be associated with elevated antioxidant status due to neutralization of peroxidant by antioxidant and free radical scavenging property of *Stevia rebaudiana* leaf (Moselhy,et al., 2016). At higher

concentration, of extract 100mg/ml the protective effect seems to be reversed towards the normal controlin the parameters studied,which could probably be associated with anti-oxidative stress and/or reduced antioxidant defense potential. This increase in activity of SOD and Catalase may be related to an increase in free radical levels due to adverse effect of high level of phytochemicals in *Stevia rebaudiana* leaf extract. Superoxide-producing lipoprotein fraction-have been reported in Stevia leaves (Isoyan,et al.,2019)

Conclusion

Indian folk medicine is replete with drugs purported to have anti-oxidative properties. The present study was undertaken to evaluate the antioxidants potential of *Stevia rebaudiana* leaf extract on red blood cells *in vitro.* Our results show a decrease in SOD and CAT activity was observed in *Stevia rebaudiana* leaf extract at concentration (0.01-10 µg/ml) as compared to the control probably due to the decrease in peroxidant mediated by antioxidant property of *Stevia rebaudiana* leaf (Milani,et al., 2017).

From our study we can conclude that the *Stevia rebaudiana* leaf extract can act as a potential antioxidant and protect the erythrocytes from oxidative damage by either directly scavenging the free radicals or activating other processes. This property of *Stevia rebaudiana* leaf may be due to various antioxidant compounds. But at higher concentration this protective effect starts to show a reversing trend probably at higher concentration. *Stevia* leaves extract itself may cause oxidative stress and /or generation of free radicals. Therefore, its indiscriminate use especially at higher concentration should be avoided. Further studies are needed to elucidate its mechanism of action and its cytotoxic property.

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Evaluation of Antibacterial Activity of Combined Plant Extract of *Pyracantha crenulata and Zanthoxylum armatum*

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DOI 10.51129/ujpah-2020-29-2(5)

Abstract-Medicinal plants represent an essential source of drugs and have played an important role in healthcare system. *Pyracantha crenulata and Zanthoxylum armatum* have been used as traditional medicine. The main aim of the study was to find the antibacterial activity of the selected plants against bacterial species: *E.coli* and *Pseudomonas aeruginosa*. The solvents used for plant extraction were hexane, chloroform, ethanol and aqueous. The in vitro antimicrobial activity was performed by Agar disk diffusion method. The hexane and aqueous extracts showed moderate activity whereas the ethanolic extracts showed a significant antibacterial activity. In the study Tetracycline was used as standard. The combined ethanolic extract of both the selected plant showed the synergistic effect on the bacterial strain tested. This leads to the conclusion that the combined effect can have possible application in the development of products as antimicrobial.

Keywords: *Pyracantha crenulata, Zanthoxylum armatum*, Antibiotics, MIC, Antimicrobial activity

Introduction

Pioneer basins of natural or herbal medicines are procured by the Medicinal plants. The structural diversity of their phytoconstituents accomplishes them as an esteemed source of novel lead compounds for the quest of drugs to treat acute and chronic diseases. Because of rich biodiversity Indian subcontinent has richest plant based traditional medicinal system. Due to better recession and lesser side effects on human body these herbal medicines are primarily used for health care. The Indian Himalayan vicinity embrace about 1,748 different species of medicinal plants.[1] The Garhwal Himalaya is acknowledged for its affluent bio-resources and ethano-culture diversity. The origin of drugs to the greatest extent depends on these natural products from plant, animal, microorganism and minerals, which are pre-owned in the therapy of human and animal disease. The extensive and lucrative use of antimicrobial medications are frequently engaged in the treatment of infectious diseases comes together with inadmissible side effects and augmentation of severe medical dilemma.[2,3] This persuades medical chemist and pharmacist to come across new antimicrobial substances from natural sources. The plant extracts screening for antimicrobial activity has demonstrated that plants are inherent origin of peculiar antimicrobial substances.[4,5] Going on the multiple drug resistance which is developed due to the extensive use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.[6,7] Antimicrobial drugs of the plant origin have immense therapeutic potential. These are virtuous in the treatment of infectious diseases while simultaneously mitigating many of the aftereffects that are much associated with synthetic antimicrobials. Growing bacterial resistance is exhorting restoration in research of the antimicrobial aspect of herbs in contrast to resistant strains.[8,9]

Zanthoxylum armatum belongs to the family Rutaceae, an important family from economic point of view. It is a wild species and locally known as Timur.[10] It is extensively dispersed in the discrete countries like India, Nepal, China, Pakistan, Bhutan, Korea, Taiwan, Laos, Myanmar, Bangladesh, Japan,

Thailand, Vietnam and Indonesia.[11] Consistently, leaves and fruits of *Zanthoxylum armatum* are used for tooth care and mouth fresh, at the same time bark is used for exhilarating the fishes and also the leaves, fruits and barks are used as spice. Commonly, the plants essential oils is used as aromatic and flavouring agents for foods and beverages.[12] In Indian medicinal Literature, *Zanthoxylum armatum* is one of the most vital medicinal plants and about 10 species are grown. Almost every part of this plant are used in the Indian traditional system for the treatment of different ailments and the compelling medicinal properties were supplementary proclaimed through scientific investigation. Plants consist of effective and active ingredients are used in therapeutics of different diseases or in the relief of pain are called medicinal plants.[13] Therapeutic and pharmaceutical role played by the plants in protecting human beings from the effects of diseases and other complications, are contemplated as to have a cogent role in the healthcare system. This is the main reason that large population of developing countries still count on the herbal medicines. Medicinal plants are taken care of in an unorganized manner and exploited for the future. Gradual increase in usage of medicinal plants has been documented over and over for both traditional users and pharmaceutical industry.[14]

Pyracantha crenulatain belongs to the family Rosaceae. It is widely acknowledged as "Ghingaroo".[15] The fruit of this plant has been used by Garhwal folk and in traditional medicines in the trietment of serious health conditions like heart disorders, hypertension, diabetes, blood pressure and circulation system especially in case of angina.[16] The leaves of plants are found useful for antioxidant, immune-modulatory, anti-inflammatory activities and are also used as herbal tea. The pome fruit of *Pyracantha crenulata* is orange-red and rich in sugar.[17] The food substances of the plant used as nutraceuticals, contain antioxidants, minerals, vitamins, prebiotics, probiotics, polyunsaturated fatty acids certain phytochemical and dietary fibers.[18]

Material and Methods

Plant materials: The leaves of *Pyracantha crenulataand and Zanthoxylum armatum* were collected from the hills of *Lohali* village located in the *Nainital District of Uttarakhand.*

Preparation of extracts: *Pyracantha crenulata and Zanthoxylum armatum* leaves were collected in a cotton bag. Cleaned and shade dried the leaves were grounded to coarse powder.

***Pyracantha crenulata* leaf extract:** The coarse powder of leaf was subjected to successive hot continuous Soxhlet extraction with different solvents (*Hexane, Chloroform, Ethanol and Water*). The liquid extract with different solvents so obtained were evaporated to get concentrated extract and stored in a closed air tight containers.

***Zanthoxylum armatum* leaf extract:** The coarse powder of leaf was subjected to cold percolation process for 7 days to obtain the extract with different solvents (*Hexane, Chloroform, Ethanol and Water*). The extracts were concentrated by evaporation and stored in a close air tight containers. Two bacterial strains were used namely *Escherichia coli* and *Pseudomonas aeruginosa*. These were cultured in nutrient broth for 24 hours and the fresh in oculums were taken for the test and reconfirmed by gram staining and sub culturing in appropriate selective media.

Anti-Bacterial Assay: The antibacterial activity was assessed by agar well diffusion method. Muller Hinton agar medium was prepared by using 15g agar dissolved in 1L distilled water. Muller Hinton agar medium was poured into each Petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. The freshly prepared in oculums were poured and spread with help of sterile glass rod all over the surface of the MHA plate. Wells of 8 mm diameter were made in the agar with a sterile cork borer. Hundred micro-liters of the working suspension/solution of different plant extracts were loaded in each well with the help of micropipette. In some MHA plates the well were poured with the combination of two plant extract of

similar solvent i.e. Hexane (*Pyracantha crenulata + Zanthoxylum armatum), Chloroform* (*Pyracantha crenulata + Zanthoxylum armatum*)**,** *Ethanol* (*Pyracantha crenulata + Zanthoxylum armatum*)**,** *and Water* (*Pyracantha Crenulata + Zanthoxylum Armatum*)**.** Plates were left for some time till the extracts diffused in the medium with the lid closed and incubated at 37°C for 24 hour. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded in Table-1

Table-1 Antimicrobial activity of plant extracts

Extract	**Concentration**	***Pyracantha crenulata***		***Zanthoxylum armatum***	
		Zone of Inhibition (Diameter in mm)			
		E. coli	***P. aeruginosa***	***E. coli***	***P. aeruginosa***
Hexane	10 mg/ml	-	12	12	11
	15 mg/ml	-	14	14	12
	20mg/ml	-	14	14	12
	25 mg/ml	-	11	14	14
Chloroform	10 mg/ml	16	12	14	16
	15 mg/ml	18	14	-	14
	20mg/ml	10	15	14	10
	25 mg/ml	14	16	12	14
Ethanol	10 mg/ml	14	22	16	16
	15 mg/ml	17	22	14	18
	20mg/ml	16	14	18	18
	25 mg/ml	18	12	18	18
Aqueous	10 mg/ml	16	16	12	11
	15 mg/ml	14	14	14	12
	20mg/ml	15	14	16	14
	25 mg/ml	17	15	14	12
Tetracycline		24	26	20	24

Results and Discussion

Antimicrobial activity of plant extracts: Preliminary antibacterial studies were conducted on selected plant extract by using two bacterial strains *E. coli* (ATCC-*11775*) and *S. aureus* (ACC-132), at concentration of 10, 15, 20 and 25 µg/ml by agar plate method. The zone of inhibition of each strain recorded for comparison using Tetracycline as standard. From the antibacterial activity data, it was found that all the extracts have shown antibacterial activity against one or the other organisms except for hexane. Ethanolic extract has shown excellent activity against both the strain. Chloroform and aqueous extract show moderate activity against both the bacterial strain in comparison to the standard Tetracycline. The antimicrobial activity of the selected plant species are summarized in Table -1.

Combinations of plant extract were performed based on the antibacterial activity of the single extracts summarized in Table-1. *Pyracantha crenulata and Zanthoxylum armatum*(50% v/v each) were mixed to evaluate in the antibacterial activity potency. The determination of the combination of *Pyracantha crenulata and Zanthoxylum armatum* ethanolic extracts is reported in Table-2. The result shows synergistic effect of using the combination of *Pyracantha crenulata and Zanthoxylum armatum* ethanolic extract.

Table-2 Antimicrobial activity of combined plant extracts

Plant Extract combination	Zone of Inhibition (Diameter in mm)	
	Escherichia coli	***Pseudomonas aeruginosa***
Pyracantha crenulata and Zanthoxylum armatum	24	26

Conclusion

In conclusion, the results of the present work provide additional information on the possible use of plant extracts, alone or in combination, in the treatment of infectious diseases. The unraveled synergistic effect of the mixture of *Pyracantha crenulata and Zanthoxylum armatum* ethanolic extracts is a proof of concept that a combination may potentiate the antimicrobial activity of each single plant extracts. In the specific case, the demonstrated improved antibacterial activity of *Pyracantha crenulata and Zanthoxylum armatum* ethanolic extracts' combination may find a possible application in the development of products for infectious diseases.

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Phytochemical and Antimicrobial Activity Screening of Stem Extracts of *Tinospora cordifolia*

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DOI 10.51129/ujpah-2020-29-2(6)

Abstract-*Tinospora cordifolia* is a popular medicinal plant which is used in several traditional medicines to cure various diseases. The common names are Amrita and Guduchi, belonging to the family of Menispermaceae. The aim of the study was to study the stem extract of traditional medicinal plant, *T. cordifolia* for qualitative estimation of phytoconsituents and subsequently to determine its antibacterial activity against two test microorganisms *Escherichia coli* and *Pseudomonas aeruginosa* to authenticate its use in traditional medicines. Stems were air-dried and coarsely powdered samples were subjected to Soxhlet extraction using diverse solvents (Hexane, chloroform, ethyl acetate and methanol and water). Freshly prepared extracts were exposed to standard phytochemical analysis for qualitative estimation of phytoconsituents. The antibacterial activity of the stem extract of *T. cordifolia* was determined by agar well diffusion method. Phytochemical analysis revealed the presence of several phytochemicals *viz.*, alkaloids, flavonoids, steroids, phenol, tannins, steroid, terpenoids, saponins and sugars. The methanolic extract displayed the presence of highest number of phytochemical compounds. It may be due to the higher solubility of active components in this solvent as compared to other solvents. The results revealed that the methanolic extract exhibit the effective antibacterial activity against the tested bacterial species . The studies justify the use of *T. cordifolia* in traditional medicines. The investigation further proposed that the metabolites present in leaf tissue of *T. cordifolia* can be potential source of novel natural antibacterial and antioxidant agents and can be prospective applications in food industry as an antioxidant.

Keywords: Antibacterial activity, Phytochemical, *Tinospora cordifolia,* Quantitative phytochemical

Introduction

Trees and plants are of supreme importance for life of humans since ancient times. Man depended on them for his physical needs such as sources for food, shelter, clothing, medicine, ornaments tools and for spiritual needs. India is a country rich in indigenous herbal resources which grow on their varied topography and under changing agro-climatic conditions permitting the growth of almost 20,000 plant species, of which about 2,500 are of medicinal value [1,2]. Peoples are now showing interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs which have adverse side effects. Medicinal plants are mostly locally available relatively cheaper and there is every virtue in exploiting such local and traditional remedies when they have been tested and proven to be non-toxic, safe, inexpensive and culturally acceptable to the community [3]. *Tinospora cordifolia* is a shady climbing shrub belonging to the family Manispermaceae, is found in the tropical areas of India, Pakistan, Sri Lanka, Burma, Africa, Australia and China.[4] The phytochemical constituents of *T. cordifolia* consists of alkaloids, sesquiterpenoid, polysaccharides, steroids, glycosides, different types of fatty acids and essential oils[4,5]. In this perspective, the plant is considered to be a nectar plant and has been called as amrita in Sanskrit in recognition of its detoxifying, rejuvenating, and immune boosting properties[6]. Experimental studies conducted on *T. cordifolia* have shown that it has significant therapeutic effects on diabetes and its associated Complications, hepatotoxicity, different types of infections, gastrointestinal related complications and different types of cancers.[7] Also, traditionally it has been reported that this plant extract has been used for the treatment of fever [4,8].

The aim of this work was to determine the phytochemical constituents of the stem extract of *T. cordifolia* and also its antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa.*

Material and Methods

Plant Collection and Processing

Stem*s* of *Tinospora cordifolia* were collected from Ranipokhari region of Dehradun(Uttarakhand) in the month of November.The botanical identity of plant was identified taxonomically . The plant material were cultivated types of 6-7 meter in height. About 20 plants both male and female were taken for study. The collected stems were cut into pieces with a sharp knife and were first washed with distilled water and disinfected for 30 minutes by immersion in a 2 % solution of sodium hypochlorite. The plant material was then rinsed with distilled water to remove residual hypochlorite. Finally the plant material was dried under shade at room temperature for 20 days. After drying plant sample was blended, pulverized and stored at 4°C. The whole experiment was conducted using a single source of dried powder of selected plant species.

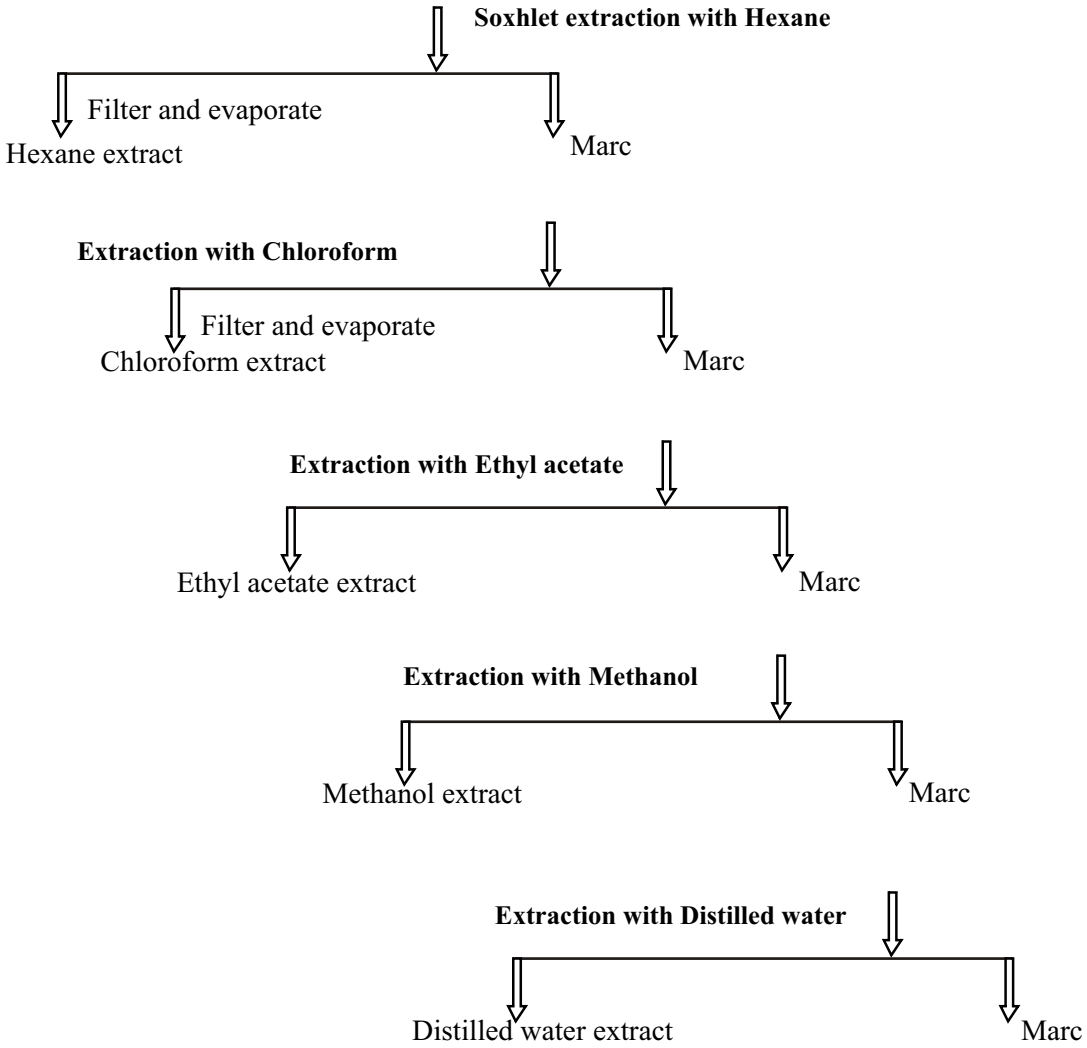
Preparation of extracts

Successive solvent extraction scheme was used for preparation of different extracts. The 500g powder of *Tinospora cordifolia* stem was used for extraction with hexane using Soxhlet's extractor. Different solvents were used for the extraction purpose in a specific sequence based on increasing polarity. Different solvents were used for dissolving different components present in the plant material, based on their different component present in the plant material, based on their different polarity. Solvents in order of increasing polarity were used.

Hexane < Chloroform < Ethyl acetate < Methanol < Distilled water

The 500g plant material was exhaustively extracted with 1000ml each of hexane, chloroform, ethyl acetate, methanol and distilled water, respectively using a soxhlet continuous extraction for 1 week. The final extract was concentrated and dried out.

Figure-1 Scheme of extraction



Marc: residue left after extraction

Determination of extractive value

The percentage extractive values (w/w) was calculated as:

$$\text{Extraction value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Phytochemical Screening

All the five extracts (Hexane, Chloroform, ethyl acetate, methanol, distilled water) were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloid, terpenoid, tannin, saponin, flavonoids, amino acids and carbohydrate. Phytochemical analysis was carried out according to following standard procedures.

1. Detection of Alkaloids: Small portion of the solvent free extract was stirred with a few drops of dil. HCL and filtered. The filtrate was then tested for the colour test to detect the presence of alkaloid in 60ml distilled water 5.0g of potassium iodide in 20ml distilled water, 20ml of distilled water) giving cream of ppt.

Hager's reagent: Test solution with Hager's reagent (saturated aq. solution of picric acid i.e. 1.0% w/w solution of picric acid in hot water) gave yellow ppt.

Wagner's reagent: Test solution with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 5ml of water and 100ml distilled water) gave reddish brown ppt.

2. Detection of Flavonoids

Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which became colourless on the addition of dilute acid, indicates the presence of flavonoids.

3. Test for Carbohydrates

Molish's test: To the 2-3 ml of extract, few drops of 95% alpha-napthol solution in alcohol were added. After shaking, conc. H_2SO_4 was added from the sides of the test tube. Appearance of violet ring at the junction of two layers indicated the positive test for reducing sugar.

Fehling's test: To1 ml Fehling A and 1 ml Fehling B reagent 1 ml of extract was added and boiled for about 10min. Formation of brick red color precipitate indicated the presence of carbohydrate[9].

Benedict's solution test: Equal volume of Benedict's reagent and extract were mixed in test tube. Heated in boiling water bath for 5 min. Appearance of red coloured solution indicates the positive test for reducing sugar.

4. Test for steroids

Liebermann-Burchard Reaction: Mixed 2ml of extract with chloroform. Added 1-2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the sides of test tube. Development of green colour reveals the positive test for steroid moiety.

Salkowiski reaction: 2ml of crude extract was dissolved in 2ml of chloroform to this added 2ml of con. H_2SO_4 sidewise, red color ring was produced[10].

5. Test for phenolic components and tanins

Small quality of test solution dissolved in water and subjected to the following tests to detect the presence of phenolic compounds and tannins.

Dil. $FeCl_3$ solution (5%) test: Test solution with few drops of ferric chloride solution showed intense green color[11-14].

Vanillin HCl acid test solution: Test solution with vanillin reagent (1gm vanillin in 10 ml concentrated HCl) gave red color.

6. Test for saponins Froth test: 2 ml of crude extract was mixed with 2ml of distilled water in a test tube, the solution was warmed and shaken vigorously; formation of stable foam indicated the presence of saponin

7. Test for protein and amino acids

Ninhydrin solution test: Heated 3ml of extract and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 min. The development of violet or purple colour showedthe presence of amino acids.[15].

Biuret test: To 3ml of aqueous extract added 4% NaOH and few drops of 1% $CuSo_4$ solution. Violet or pink colour is formed, if proteins are present.

Thin layer chromatography profiling of various extracts

Nowadays, TLC is employed as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations[16-18]. The TLC profiling was performed as per described by Biradar et al., 2013[19]. Various extracts of stem of *Tinospora cordifolia* were obtained by sequential Soxhlet extraction with different solvents of increasing polarity. 100gm of powdered sample were sequentially extracted in a Soxhlet extractor using 800 ml of hexane, chloroform, ethyl acetate, methanol and distilled water, The extraction was done until solvent in soxhlet became colourless. The extracts were then subjected to distillation for preparation of crude extracts in respective solvents. The TLC plates were prepared by using Silica gel 'G'

as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 mins and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts of hexane, chloroform, ethyl acetate, methanol and distilled water, with respective solvent and then applied usually 1-10µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes. After the application of the sample on the plate, the plates were kept in TLC glass chamber (solvent saturated) then mobile phase was allowed to move through adsorbent phase up to 3/4th of the plate. TLC was performed for alkaloids, flavanoids, tannins and phenols, solvent system (Table-2)

Assessment of antibacterial activity

Antibacterial activity of *T. cordifolia* stem extract was carried out against *Escherichia coli* and *Pseudomonas aeruginosa, accourdingto the* method of Wang *et al*., (2012)[20]. Aqueous, methanolic, ethyl acetate, chloroform and hexane extracts were used as test compound and ampicillin was used as reference standard. The Petri dishes were thoroughly washed and sterilized in hot air oven at 160ºC for one hour. The sterile plates were previously labelled with description and date. To each petri plate, 15 to 20 mL of liquefied sterilized nutrient agar was transferred and allowed to cool and set. The bacterial cultures were activated prior to the experiment. Soft agar containing target cultures (200 µL) were overlaid. The wells were made with the help of sterile well borer (6 mm). The test solution (0.1ml) was added to the respective bores .Finally, petri plates were kept for proper diffusion and overnight incubated at 37°C for 24 hours. At the end of the incubation period, zone of inhibition was observed and measured using a scale[21].

Results and Discussion

Extraction Value and Color of Plant Extract

The plant material was exhaustively extracted with each of Hexane, chloroform, ethyl acetate and methanol respectively using a Soxhlet continuous extraction apparatus for 1 week. The final extracts were concentrated and dried whose colour, nature and %age yield has been shown in Table-1.

Table -1 The percentage extractive values (w/w) along with color of the extract and nature of the residue is presented below

S .No.	Solvent	Colour of the extract	Natural of residue	Extraction value%(w/w)
1	Hexane	Green	Solid	8.3
2	Chloroform	Brown	Solid	25.23
3	Ethyl acetate	Brown	Semi solid	11.44
4	Methanol	Yellow	Solid	10.34
5	Distill water	Dark brown	Semi solid	11.50

The percentage extractive values (w/w) for Hexane, Chloroform, Ethyl acetate, Methanol and Distilled water was found to be 8.3, 25.23, 11.44, 10.34 and 11.50 respectively (Table-1)

Phytochemical Screening

Phytochemical screening of the sequential extract of *Tinospora cordifolia* revealed the presence of various bioactive components. The test for alkaloid has given positive result whereas saponin and protein test showed negative result for all the four extracts taken under study. Carbohydrates were present only in chloroform extract, amino acid in aqueous extract and flavonoid in ethyl acetate extract.

The result of phytochemical test is presented in Table -2.

Table-2 Shows qualitative phytochemical analysis of extracts of *Tinospora cordifolia*

Phytochemical Test	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract
Test for flavonoids				
Alkaline reagent test	+	-	+	-
Test for Alkaloids				
Mayer's test	+	+	+	+
Hager's test	+	+	+	+
Wager's test	+	+	+	+
Test for carbohydrates				
Fehling test	+	+	+	+
Molish test	-	+	-	+
Benedict's test	-	+	-	+
Test for phenolic compounds and tannins				
Dil. $Fecl_3$-test	+	+	-	+
Vanillin - HCl Test	_	_	+	+
Test for steroids				
Salkowiski test	-	-	+	+
Test for saponins				
Froth Test	-	-	-	-
Test for proteins				
Ninhydrin	-	-	-	-

(-) A sign indicates absence of constituent in the respective screening test;
(+) sign indicates the presence of a constituent in the respective screening test.

These results are in confirmation with earlier studies done for this plant[22,23]. Flavonoids have extensive biological properties that promote human health and help in reduction of risk of diseases due to their antioxidant, anticancer, anti-inflammatory and anti-microbial properties[24]. Tannins are basically cytotoxic agents. They act as free radical scavengers thus they can be useful in treatment of various degenerative diseases like cancer, atherosclerosis, and aging process[25]. Alkaloids are being used in life saving drugs for some critical disorders like cancer, heart failure, blood pressure due to their wide range of pharmacological activities[26]. Saponins have been considered as bioactive antibacterial agent but also act as anti-tumour agents by inducing apoptosis [27]. Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. From the above results, it can be noted that successful extraction of biologically active compounds from plant are largely dependent on the type of solvent used during extraction. In this study, different solventswere used. This study, therefore, validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract[28].

Thin Layer Chromatographic studies (TLC)

Thin layer chromatography was carried out on silica gel G plates made manually in laboratory. The samples were loaded 2 cm above from the bottom of the plates with the help of micropipettes to uniformly apply the samples and allowed to dry. The plates were developed in a chromatography chamber using different solvent systems according to the extract.For detection of flavonoids solvent system consisted of ethyl acetate: formic acid: water (40: 5: 5) likewise for the detection of alkaloids the solvent system consisted of methanol: ammonia (20: 3) respectively. For the identification boric acid and oxalic acid mixture were used for flavonoids and Mayer's reagent was used.The plates were air dried and then kept in hot air oven at 100 °C for 5-6 minutes and then were observed and visualized under visible light followed by spraying with 10% H_2SO_4 and then again the plates were visualized under UV . The retention factor (Rf values) for each active compound was calculated .

Table - 3 R_f value of different extract of *Tinospora cordifolia* stems extract

S. No	*T.cordifolia* Extracts	Solvent System Used	No of spot	R_f value
1	Hexane	Chloroform:Methanol (20:7)	2	0.1,0.3
2	Chloroform	Toluene:Chloroform:Methanol(4.5:5:0.5)	4	0.1,0.2,0.4,0.7
3	Ethyl acetate	Cyclohexane:Formic acid (6:1)	3	0.2,0.6, 0.8
4	Methanol	Hexane: Ethyl acetate (9:1)	1	0.8
5	Distill water	Butanol:Aceticacid:Water (4:5:1)	1	0.4

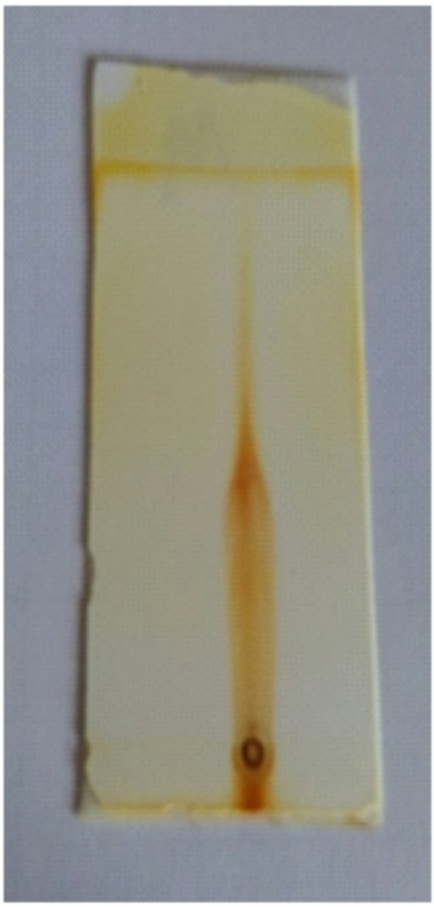


Figure-3
TLC of Methanol extract

Figure- 4
TLC Of chloroform extract

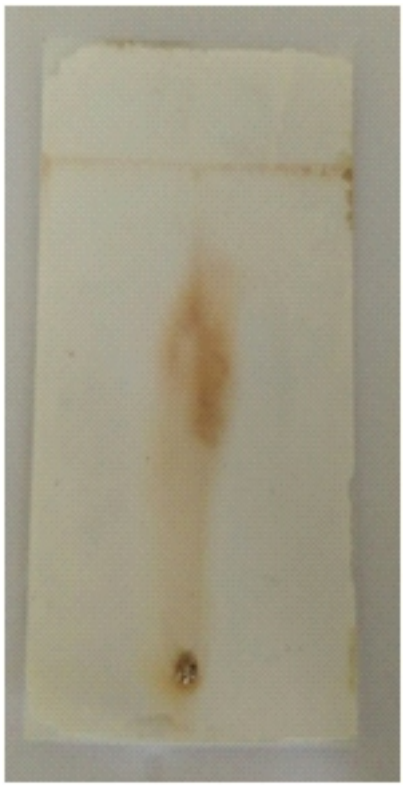


Figure-5
TLC of Ethyl acetate

Antibacterial activity of *T. cordifolia* was recorded against *E. coli* and *Pseudomonas aeruginosa*. All the four extracts showed antibacterial activity against the two target bacterial species under study, although to a varying degree, as shown in Table-4. The zone of inhibition also differed for the same extract against different bacterial species. The activity was measured in terms of zones of inhibition in diameter (mm) for methanolic, ethanolic, chloroform, hexane and acetone extracts of Stem tissue (Table-4).

The results revealed that the methanolic extract exhibit the effective antibacterial activity against the tested bacterial species.Antimicrobial activity of the *T. cordifolia* with different solvents on different micro-organism showed good antifungal and antibacterial activity[29]. Available reports tend to show that secondary metabolites such as alkaloids, flavonoids, tannins, and other compounds of phenolic nature are responsible for the antimicrobial activities in higher plants[30].

Table-4 Anti- microbial activity of stems extract of *Tinospora cordifolia* agains different bacteria strains:

Organism used		Zone of inhibition(mm)												
		Hexane Concentration			Chloroform Concentration			Ethyl acetate Concentration			Methanol Concentration			Ampicillin
		A	B	C	A	B	C	A	B	C	A	B	C	25(µg/ml)
1.	*E. coli*	8.7	10	11.6	8.5	9.1	10.3	10	12	13.6	14.2	15	17.5	25.8
2.	*Pseudomonas aeruginosa*	5	6.5	7.0	6	6.4	7.4	11	11.5	12	11.2	12.1	13	20.4

A: Dose 50 mg/ml B: Dose 75 mg/ml C: Dose 100 mg/ml

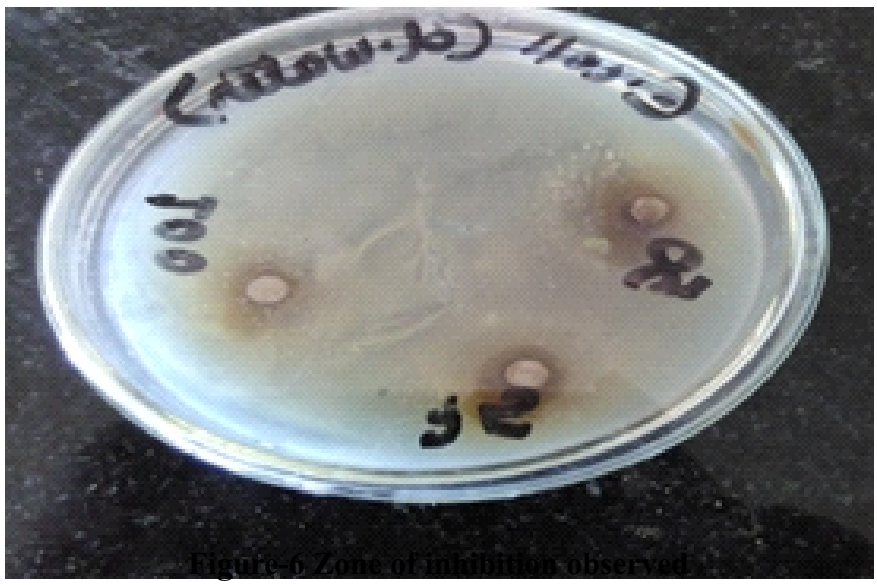


Figure-6 Zone of inhibition observed against *Escherichia coli*(Distil water extract)

Conclusion

Herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and mainstream system. India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plants based formulations, used in folk medicines and known to rural communities in India. Medicinal plants play a central role not only as traditional medicines, but also as trade commodities.

In the present work phytochemical and antimicrobial investigation of *Tinospora cordifolia* was performed. Successive solvent extraction was done using soxhlet. Preliminary phytochemical screening of *T. cordifolia* gave valuable information about the different phytoconstituents present in the plant. It showed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins and amino acids. This will help the future investigators concerning the selection of the particular extract for further investigation of isolating the active principles. It gave idea about different phytochemicals which have been found to possess a wide range of activities. *T. cordifolia* stem extracts exhibited marked dose dependent antimicrobial activity in vitro against the two bacteria *E. coli* and *Pseudomonas aeruginosa* which can be used as a good therapeutic approach for infectious disease management and therapy. Methanolic extract was found to be more potent against both the group of bacteria. *T. cordifolia stem* has shown different types of phytochemicals. Methanolic extract of *T. cordifolia* stem exhibited better antioxidant potential also. Further purification, quantification and antioxidant potential of the active compounds would be our priority in future studies. Both in vitro and in vivo studies are recommended for therapeutic applications in modern medicine.

Declaration

Current research work is not funded by any funding organisation/Agency.

Conflict of Interest

There is no any conflict of Interest among any author.

Acknowledgment

The authors are highly thankful to the Department of Biochemistry and Biotechnology, Sardar Bhagwan Singh University,Balawala, Dehradun(Uttarakhand) for providing all help and required facilities to carry out the research.

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Comparative Analysis of Gluten Content of Raw and Processed Materials

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DOI 10.51129/ujpah-2020-29-2(7)

Abstract-Gluten intolerance /allergies is now a silently rising problem world-wide. Gluten is a protein composite found in cereals, wheat, rice, barley and certain oat varieties. It also causes Celiac disease which is an autoimmune disease (most severe form of gluten intolerance), resulting from gluten intolerance and is based on genetic intolerance. The only therapeutic treatment for the patients with gluten allergies and celiac disease is a strict gluten free diet. Rising demand for gluten free products is a task for the bakers and manufacturers to eliminate gluten completely from their products which is technically not possible. This draws the attention to the fact that mandatory analysis in this field is required. The aim of this work is to find a way to reduce the gluten content in the available materials, and also to find the source of gluten contamination after processing of raw materials. It was noted that garlic and sugar containing combination were found effective and showed decrease in gluten content and that they were better additivities in the processing process than rest of others.

Keywords: Gluten intolerance, autoimmune disease, Celiac disease, Gluten contamination, Allergies.

Introduction

Gluten is a naturally found protein mixture of prolamin and glutelin proteins present in wheat, barley, rye and other grains (Janssen, A. M., et. al.1996).Gluten comprises 7585% of the total protein in bread wheat. Wheat gluten is composed of mainly two types of proteins, the glutenins and the gliadins.(St. Paul, US 2001)

Gluten helps food maintain their shape, acting as a glue that holds food together. It's what makes dough elastic and gives bread its chewy texture (Brevis, J. C., Morris, et al).

These properties, and its relatively low cost, make gluten valuable to both food and non-food industries.(C. Wrigley,et al). Apart from its use in industries and food products it is also responsible for allergies mainly celiac disease.

Celiac disease is an autoimmune disorder that's triggered when you eat gluten. It is also known as celiac sprue, nontropical sprue, or gluten-sensitive enteropathy. (Briani, C. et.al. (2008).

When someone with celiac disease eats something with gluten, their body overreacts to the protein and damages their villi, small finger like projections found along the wall of their small intestine. Damaged villi can lead to malnourishment as well as loss of bone density (L.Augustin, et al 2006).Symptoms include- Abdominal pain, Anemia, bloating, bone or joint pain, constipation, diarrhea, gas, heartburn, itchy blistery rash, headache or fatigue, mouth ulcers, nausea, weight loss, nervous system injury, miscarriage, infertility or even neurological disease or certain cancers, all this due to inability of your small intestine to absorb nutrients from food(St. Paul, US 2001).

Most of the people with celiac disease never know that they have it. Only few as 20% of people get the right diagnosis. The damage to your intestine is very slow, and symptoms are so varied that it can take years to get a diagnosis.

Gluten content in the test samples were analysed by Enzyme Linked Immunoassay (ELISA) using ELISA kit from r- biopharm.

It is first discovered by Eva Engvall and Peter Perlman. Antigen from the sample to be tested are attached to a surface. Then a matching antibody is applied over the surface, so it can bind the antigen. This antibody is linked to an enzyme and then any unbound antibodies are removed. In the final step, a substance containing the enzyme's substrate is added. If there was binding the subsequent reaction produces a detectable signal, most commonly a colour change (D., Sidney, et al (2009). The assay uses a solid-phase type of enzyme immunoassay (EIA) to detect the presence of a ligand commonly protein in a liquid sample using antibodies directed against the protein to be measured. ELISA has been used as a diagnostic tool in medicine, plant pathology. Biotechnology, as well as a quality control check in industries. (Ehren, J., Govindarajan, et al 2008).

Material and Methods

Samples of wheat flour and maida was collected from shop. For processing Additives-powders (ginger,garlic, fenugreek, ajwain,and sugar), water,

milk, butter milk and oil were used. (Goesaert, H.et al.2005). Different combinations (table-1) A, B, C, D, E, F, G, H, I, J, K, L, M were prepared -using additives for the test. Samples were prepared according to the provided protocol of the ELSA test kit. Prepared samples were loaded in microwells and incubated for 30 min's. The wells of microtiter strips are coated with R5 antibodies against Gliadins. By adding the standards or sample solution to wells, present gliadin will bind to the specific capture antibodies. The result is antibody-antigen complex. Components not bound by the antibodies are then removed in a washing step. Then R5 antibody conjugated to peroxidase is added to the wells and incubated. Bound conjugate converts the colorless chromogen into a blue product. The addition of the stop solution leads to a colour change from blue to yellow. The measurement is made photometrically at 450nm. The absorbance is proportional to the gliadin concentration of the sample (RIDACREEN® Gliadin Art.Nr. r7001 manual).

Results and Discussion

Gluten content in wheat samples after being processed was found to be near the value of raw wheat flour in most of the samples that is greater than 80, but some combination of samples **F** and **I** showed a remarkable fall in the gluten content after processing. Sample **I** containing garlic and **F** containing sugar showed value less than 80mg/kg (table-1).

In case of Maida- it was found that gluten content was 67.93 mg/kg in raw form which get increased upto 10-11mg/ kg after processing. Gluten content was found highest in combination containing oil that is 77.59, (table-1).

Table-1

S.NO	SAMPLE NAME	SAMPLE CODE	GLIADIN (mg/kg)	GLUTEN CONTENT (mg/kg)
1	Wheat flour (dry)	A	>40	>80
2	Wheat flour + Water	B	>40	>80
3	Wheat flour + Milk	C	>40	>80
4	Wheat flour + Butter Milk	D	>40	>80
5	Wheat flour + Oil + Water	E	>40	>80
6	Wheat flour + Garlic + Water	F	**39.21**	**78.43**
7	Wheat flour + Ginger + water	G	>40	>80
8	Wheat flour + Ajwain + water	H	>40	>80
9	Wheat flour + Sugar	I	**36.97**	**73.94**
10	Wheat flour + Methi	J	>40	>80
11	Maida(dry)	K	33.97	67.93
12	Maida flour + Water	L	34.78	69.57
13	Maida + Oil + Water	M	38.79	77.59

- In wheat flour samples **F** and **I** decrease in gluten content was observed.
- In maida samples anrise in gluten content was observed from K to M

Conclusion

1. Garlic (F) and sugar (I)containing combination were found effective and showed decrease in gluten content. Hence from our study and results we can conclude that garlic and sugar were better additives in the processing process than rest of the others. Both can be used in lowering/processing the gluten content in wheat flour.
2. Conclusion drawn from maida samples, oil, tends to increase gluten content in maida, it's not recommended for processing/lowering gluten.

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CCl_4 Induced Hepatotoxicity and It's Recovery by Leaves and Seed Extract of *Cassia Fistula*

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DOI 10.51129/ujpah-2020-29-2(8)

Abstract-The present communication is based on herb, namely, *Cassia fistula*. belonging to family Fabaceae (Caesalpiniaceae) for the prevention of intoxication induced with a sublethal dose of Carbon tetrachloride (CCL_4). Male mice with a dose weighing around 6.5 mg/kg body weight were exposed to CCl_4, a common toxic agent used for hepatotoxicity. Seed and leaves extracts of *Cassia fistula* were given thereafter to the male mice to check their effect on the toxicity of CCl_4. After several weeks of exposure to these herbal extracts, there was a significant change observed in the biochemical parameters with a significant increase in values of serum oxaloacetatate transferase, serum glutamate pyruvate transferase, gamma-glutamyl transferase, alkaline phosphatase, creatine kinase, creatinine, glucose, and cholesterol. In contrast, no significant statistical difference was perceived in the values of all biochemical parameters in mice treated with leave and seed extracts, except the value of serum cholesterol in mice treated with seed extract which decreased as compared to the control group. These findings suggest the importance of the seeds and leaves and their combined usage against CCl_4 toxicity, thus promising these plant products to be promising therapeutic agents against hepatotoxicity, cardiotoxicity, nephrotoxicity, and metabolic disorders due to CCl_4 toxicity. This also makes the safety of herbal products an important public health issue.

Keywords: Seeds and leave extract of *Cassia fistula*, CCl_4, Biochemical Parameters, Hepototoxicity.

Introduction

India is one of the richest countries among the world in resources of medicinal plants in various sytems like Ayurveda, Siddha, Unani, etc[1,2]. The treatment of patients with these medicinal plants is increasing from previous to till dates[3]. The all medicinal plants contain several biochemical or chemical active substances that show a definite physiological action on human or animal body while applying as Treatment[4]. Some literature show the value and emerging effect of medicinal plant in Ayurveda. In Ayurvedic medicine, the golden shower tree is known as aragvadha, meaning disease killer[5]. The plant aragvadha all species are native to the Indian subcontinent and adjacent regions of Asia, South Africa, Mexico, East Africa and Brazil. It is found in worldwide but it is known as national tree of Thailand and its flower is national flower of Thailand. In India, it is the also state flower of Kerala and of immense importance amongst the Malayali population[6]. *Cassia fistula* is widely grown as ornamental profusely yellow flowering tree in tropical area which blooms in late spring but many times no leaf being seen. A tree 6-9 m high; trunk straight; bark smooth and pale grey when young, rough and dark brown when old; branches spreading, slender. The leaves are deciduous and pinnate with three to eight pairs of leaflets. The flowers are pendulous racemes, slender, pubescent and glaborous 47 cm diameter[7]. Calyx long divided to the base, pubescent; segments oblong, obtuse, corolla yellow, stamens all antheriferous. The fruit is legume with a pungent odor and containing several seeds. The long pods green pods turn black on ripening after flowers shed[8,9]. Pulp is dark brown in colour, sticky, sweet and mucilaginous, odour characteristic, and somewhat disagreeable[10,11]. Drug occurs in flat or curved thick pieces; outer surface smooth to rough with warty patches; greenish grey to red; inner surface rough, reddish with parallel striations; fracture, laminate; odour, sweet and characteristic; taste, astringent[12]. The pods are pendulous, with numerous (40-100) horizontal seeds immersed in a dark coloured sweetish pulp. Seeds are broadly ovate, slightly less in breadth and thick[13]. The plant is widely planted as tree and has a number of common names in various languages from its native range surrounding regions such as in English (golden shower cassia, Indian Laburnum, golden shower, pudding pipe tree, purging cassia, purging

Fistula.The fruit and seed pulp and root have immense medicinal value. It is prescribed as emetics, purgatives, febrifuges and relievers of thoracic congestion[12,13,14]. It used in reliving asthma, leprosy, ringworm, fever and heart related diseases. In Ayurvedic medicine system, seed used as antibilious, aperitif, carminative and laxative, root for adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands, leaves for erysipelas, malaria, rheumatism and ulcers, the buds for biliousness, constipation, fever, leprosy and skin disease, the fruit for abdominal pain, constipation, fever, heart disease and leprosy [15]. In Unani system, leaves used for inflammation, flowers as purgative, the fruit as antiinflammatory, antipyretic, abortifacient, demulcent, purgative, refrigerant, good for chest complaints, eye ailments, flu, heart and liver ailments, and rheumatism, though suspected of inducing asthma[16] . The entire plant or plant parts are used by several ethnic groups for their tradation, rituals and to cure several diseases from their inherent knowledge. Plant has bright yellow flowers in spring and summer in northern India[17]. There was so many research documented of the hepatoprotective effect of *Cassia fistula*[18,19,20]. Some literature showed the value and emerging effect of hepatoprotective activity of leaf extracts of the *Cassia fistula* [19, 20]. In view of the above literature this study was planned to screen the hepatoprotective activity of both leaves and seed extract of *Cassia fistula.*

Meterial and Methods

Experimental animals

The experiments were done using male albino mice weighing 2431 g. The mice were obtained from the experimental animal house of the institute. The principles and rules of laboratory animal care were followed during all the experiments. The experimental animals were housed in standard polypropylene cages and maintained under controlled laboratory conditions of humidity (65%), temperature (25±1°C), and 12:12 h light:dark cycle, with balanced food and water. They were initially acclimatized for the study, and the study protocol was approved by the Institutional Animal Ethics Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals, New Delhi.

Leaves and seeds extraction

The new leaves normally appear during March-July and flowers in April to July in India. The long cylindrical pods develop rapidly and reach their full length by October and they ripen during December-March and ripe pods in May. The fresh young leaves and seeds of *Cassia fistula* were directly collected from some tree plantation farms in Hills of Dehradun, Uttarakhand. The leaves and seeds were thoroughly washed and dried at room temperature. The fine quality of dried leaves was kept in a dry plastic container until being used for extract preparation. The collected seeds were rendered free from soil and impurities manually were kept in a dry container until being used for extracts. The dried leaves (10 g) and seeds (30 g) were powdered separately and added to 500 ml cold water and mixed in an electric mixer for 10 min. Thereafter, the solution of leaves and seeds were filtered, and the filtrates were evaporated in an oven at 40°C to produce dried residues (active principles). With references to the powdered samples, the yield of the tea extract was 16.1%. Furthermore, the extracts were weekly prepared and stored in a refrigerator for subsequent experiments. Plant leaves and seeds were then authenticated by Mr. A. Sundriyal, Faculty at Department of Pharmacy, SBS University, Dehradun. While the identification completed, each sample was deposited in the SBS University, School of Pharmacy Dehradun, herbarium with individual voucher number.

Experimental treatments

A total of 48 mice were randomly divided into eight experimental groups of six mice in each group. The experimental groups were treated as follows [18].

1. Mice of Group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), 5 times weekly, for 7 weeks.

2. Mice of Group 2 were intraperitoneally given CCl_4 at the level of 6.5 mg/kg body weight (1/10 of LD 50), 5 times weekly, for 7 weeks.

3. Animals of Group 3 were orally supplemented with leaves extract of *Cassia fistula* (400 mg/kg body weight) and after 4 h received CCl_4 at the same dose given to Group 2, 2 times weekly, for 7 weeks.

4. Mice of Group 4 were orally supplemented with seed extract of *Cassia fistula* (400 mg/kg body weight) and after 4 h received CCl_4 at the same dose given to Group 2, 5 times weekly, for 7 weeks.

5. Animals of Group 5 were orally supplemented with seed and leaves extract of *Cassia fistula* (200 mg/kg body weight) after 4 h received CCl_4 at the same dose given to Group 2, 5 times weekly, for 7 weeks.
6. Mice of Group 6 were intraperitoneally received saline solution at the same dose given to Group 1 and were orally supplemented with leaves extract of *Cassia fistula* at the same dose given to Group 3, 5 times weekly, for 7 weeks.
7. Mice of Group 7 were intraperitoneally received saline solution at the same dose given to Group 1 and were orally supplemented with seed extract of *Cassia fistula* at the same dose given to Group 4, 5 times weekly, for 7 weeks.
8. Animals of Group 8 were intraperitoneally received saline solution at the same dose given to Group 1 and were supplemented with tea and seed extracts of *Cassia fistula* at the same dose given to Group 5, 5 times weekly, for 7 weeks.

Biochemical analysis

After 7 weeks, mice were anesthetized with diethyl ether. Blood was collected from orbital venous plexus in plain gel tubes and centrifuged at 2500 rpm for 10 min, and serum were then collected and stored at 4°C till the determination time of serum glutamate oxalo transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), creatinine, glucose, total protein, triglycerides, and cholesterol by using Semi-Automated Clinical Chemistry Analysis System, ERBA chem-7.

Statistical analysis

Data were expressed as the mean±standard deviation and were analyzed by one-way analysis of variance using the Statistical Package for Social Sciences (SPSS for Windows, version 12.0). Multiple comparative analyses were conducted between all experimental groups using Tukey's test. Results were considered statistically significant at $p<0.05$.

Results and Discussion

Male albino mice exposed to sub-lethal doses of CCl_4 were treated with *Cassia fistula* leaves, and seed extracts, and their combination, which showed varied levels of biochemical markers in mice serum. Mice treated with CCl_4 (Group 2) showed a significant increase in the values of serum SGOT (84.21±4.21 IU/L), SGPT (106.61±12.10 IU/L), GGT (25.17±3.26 IU/L), ALP (235.80±44.9 IU/L), CK (620.01±25.11 IU/L), creatinine (2.83±1.14 mg/dl), glucose (162.17±36.44 mg/dl), total protein (4.83±0.26 g/dl) triglycerides (172.20±28.51 mg/dl), and cholesterol (201.8±28.1 mg/dl) when compared to control mice (Group 1). The leaves extract of *C. fistula* showed decrease in the values of serum SGOT (41.12±1.12 IU/L), SGPT (50.61±7.21 IU/L), GGT (13.11±2.22 IU/L), ALP (151.34±20.11 IU/L), CK (411.20±22.02 IU/L), creatinine (1.43±0.90 mg/dl), glucose (120.33±20.30 mg/dl), total protein (5.21±0.30 g/dl) triglycerides (98.67±14.6 mg/dl), and cholesterol (140.11±12.37 mg/dl). However the level of these all biochemical parameters are decreased in the group of mice treated with leaves extract of *C. fistula* while compare with treated group of mice with CCl_4 (Carbon tetrachloride) and treated with seed extract of *C. fistula* plus CCl_4. Other treated groups (Tables 1 and 2). However, the level of total serum protein statistically declined in Group 2 treated mice when compared to control and other treated group mice (Table 2). On comparison with the control mice, the mice treated with tea leaves extract plus CCl_4 (Group 3), seed extract plus CCl_4 (Group 4), and leaves and seed extracts of *C. fistula* plus CCl_4 (Group 5) showed a significant increase in values of serum ALT, AST, GGT, ALP, CK, creatinine, glucose, and cholesterol. In addition, some non-significant changes in serum total protein were observed in mice of Groups 3, 4, and 5. The triglycerides level statistically increased in mice treated with leaves extract plus CCl_4 (Group 3), seed extract plus CCl_4 (Group 4), while the levels remain unchanged in mice supplemented with leaves and seed extracts plus CCl_4 (Group 5). In addition, no significant statistical difference was perceived in the values of all biochemical parameters in mice treated with leave extract (Group 6), seed extract (Group 7), and leave and seed extracts (Group 8) except the value of serum cholesterol in mice treated with seed extract (Group 8) which decreased as compared to the control group. Together, the above results indicate that the toxic effect of CCl_4 was much decreased and attenuated with the combinatorial effect of leave and seed extracts of *C. Fistula*.

The values of routine biochemical markers such as

serum ALT, AST, GGT, ALP, CK, creatinine, glucose, triglycerides, and cholesterol were significantly higher, while low serum protein was observed with mice which were exposed only to CCl_4. These results were in concordance with observations of the previous studies which also showed severe alterations in physiological and Biochemical parameters in experimental animals[19,20]. The increase in the levels of ALP, ALT, AST, and GGT seen here indicated liver dysfunction, and these markers have been in use for biochemically monitoring chemical-induced tissue damage [21]. In addition, reports also confirm the release of these biomarkers in bloodstream when the hepatic parenchymal cells are exposed to toxic pesticides like CCl_4 (Carbon tetrachloride)[19,20,21]. These observations were supported by Anil *et al.* in different studies which also showed cardiotoxicity due to paracetamol exposure in experimental animals[18]. This increase indicates the inhibitory action of pesticide on cytochrome P450 enzymes. Furthermore, increased cholesterol concentration indicates liver disorders and cholestasis[19]. *C. fistula*, the traditional plant is proven to have a high content of flavonoids which are phenolic compounds of plants and have been attributed for several functions including many health benefits. These flavonoids are potent antioxidants, and metal chelators which protect cells from reactive oxygen species or free oxygen radicals are a few among Many other good studies which have shown the protective effect of green tea on pesticides and organophosphorus compounds [15,16,19,20,21]. In addition, this study also explored the use of seed extract which has also been studied in the prevention of decrease in antioxidant levels and having an ameliorative effect on hematological, biochemical, and histopathological alterations.

Conclusion

This study is an attempt toward showing the promising role of extracts of *C. fistula* in preventing hepatotoxicity, cardiotoxicity, nephrotoxicity and metabolic disorders induced due to CCl_4. Furthermore, there is a need of biochemical, histopathological, and physiological investigations using different dosages of these crude extracts to prove the plausible role of these extracts in preventing CCl_4 toxicity. In addition, the combinatorial effect of these extracts might also be explored to study their effect on other toxicants and pathogenic moieties.

Table-1 Serum of control and treated mice (with CCl_4, CCl_4+ leave extract of *C. fistula*, CCl_4+ seed estract of *C. fistula*, CCl_4+ leaves and seed extract of *C. fistula*, *C. fistula* leave extract alone, *C. fistula* seed extract alone, and combination of leaves and seed extract of *C. fistula*) showing different concentrations of SGOT, SGPT, GGT, ALP and CK.

S. No	Treatments	Parameters				
		SGOT (U/L)	SGPT (U/L)	G-GT (U/L)	ALP (U/L)	CK (U/L)
1	Control	22.66±1.12	33.33±2.11	6.82±4.21	102.00±4.32	300.5±7.12
2	CCl4	84.21±4.21^{ab}	106.61±12.10^{ab}	25.17±3.26^{ab}	235.80±44.9^{ab}	620.01±25.11^{ab}
3	leave extract of *C. fistula*+CCl_4	41.12±1.12^{a}	50.61±7.21^{a}	13.11+2.22^{a}	151.34±20.11^{a}	411.20±22.02^{a}
4	seed extract of *C. fistula*+CCl_4	44.32±1.21	60.22±12.26^{a}	14.12±2.22^{a}	158.26±38.20^{a}	442.67±16.21^{a}
5	leave extract and seed extract of *C. fistula*+CCl_4	33.20±2.01^{a}	46.60±6.22^{a}	7.00±1.21^{a}	144.61±22.71^{a}	401.22±17.60^{a}
6	leave extract extract of *C. fistula*	23.5±1.72	34.71±3.22	6.24±1.26	114.6±12.12	301.31±22.15
7	Seedextract of *C. fistula*	25.21±1.41	35.41±2.11	7.31±1.82	119.40±8.2	318.24±20.11
8	leave extract+ seed extract extract of *C.fistula*	24.33±1.21	36.42±3.26	6.30±0.56	110±8.4	292.31±14.12

Data represents the means ±SD of 6 animals per group. [a]Indicates a significant difference between the control and treated groups. [b]Indicates a significant difference between mice exposed to CCL_4 and *C. fistula* leaves extract + CCl_4, *C. fistula* seed extract plus CCl_4, leaves and seeds extracts *C. fistula* plus CCl_4, *C. fistula* leave extract, *C. fistula* seed extract and leave and seed extracts *C. fistula* treated mice.

Table-2 Serum of control and treated mice (with CCl_4, CCl_4+ leave extract of *C. fistula*, CCl_4+ seed estract of *C. fistula*, CCl_4+ leaves and seed extract of *C. fistula*, *C. fistula* leave extract alone, *C. fistula* seed extract alone, and combination of leaves and seed extract of *C. fistula*) showing different concentrations of creatinine, glucose, total protein, triglyceride and cholesterol.

S. No	Treatments	Parameters				
		Creatinine **(mg/dl)**	Glucose **(mg/dl)**	Total protein **(g/dl)**	Triglyceride **(mg/dl)**	Cholesterol **(mg/dl)**
1	Control	0.67±0.14	82.12±8.3	5.18±0.43	82.24±4.65	94.80±4.22
2	CCl4	2.83±1.14^{ab}	162.17±36.44^{a}	4.83±0.26^{ab}	172.20±28.51^{ab}	201.81±28.1^{ab}
3	leave extract of *C. fistula*+CCl_4	1.43±0.90^{ab}	120.33±20.30^{a}	5.21±0.30	98.67±14.6^{a}	140.11±12.9^{a}
4	seed extract of *C. fistula*+CCl_4	1.53±0.33^{a}	124.50±11.2^{a}	5.42±0.22	108.60±16.8^{a}	150.01±12.37^{a}
5	leave extract and seed extract of *C. fistula*+CCl_4	1.34±0.48^{a}	148.67±24.5^{a}	5.13±0.29	94.00±5.9^{a}	116.66±8.46^{a}
6	leave extract extract of *C. fistula*	0.68±0.12	85.11±7.23	5.16±0.21	88.00±4.46	96.22±6.22
7	seed extract of *C. fistula*	0.76±0.18	94.93±6.99	5.15±0.32	77.81±4.1	98.46±6.2^{a}
8	leave extract+ seed extract extract of *C. fistula*	0.64±0.14	86.0±0.87	5.13±0.33	80.11±6.4	96.11±11.22

Data represents the means ±SD of 6 animals per group. [a]Indicates a significant difference between the control and treated groups. [b]Indicates a significant difference between mice exposed to CCL_4 and *C. fistula* leaves extract + CCl_4, *C. fistula* seed extract plus CCl_4, leaves and seeds extracts *C. fistula* plus CCl_4, *C. fistula* leave extract, *C. fistula* seed extract and leave and seed extracts *C. Fistula* treated mice. Data represents the means ±SD of 6 animals per group. [a]Indicates a significant difference between the control and treated groups. [b]Indicates a significant difference between mice exposed to CCL_4 and *C. fistula* leaves extract + CCl_4, *C. fistula* seed extract plus CCl_4, leaves and seeds extracts *C. fistula* plus CCl_4, *C. fistula* leave extract, *C. fistula* seed extract and leave and seed extracts *C. fistula* treated mice.

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Quantitative Determination of Aloin in *Aloe vera* and Its Antioxidant Activity

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DOI 10.51129/ujpah-2020-29-2(9)

Abstract-*Aloe vera* is a popular medicinal plant used widely by the cosmetic, pharmaceutical and food industries. The *Aloe vera* gel, which is used mostly for its positive effects on human health, contains over 75 different bioactive compounds, including aloin. A sensitive and reliable densitometric High Performance Thin Layer Chromatography method has been developed for the quantification of aloin, an anthraquinone present in *Aloe vera* leaves. Chromatographic analysis was performed using methanol extract of leaves of *Aloe vera* using solvent system ethyl acetate: Methanol: water (100:13.5:10). Detection and quantification of aloin was done by densitometric scanning at 350nm. The results of linearity range and correlation coefficient show that there was a good correlation between peak area and corresponding concentration of aloin. The method developed here in can be implemented in the analysis and routine quality control of herbal materials. Antioxidant activity of *Aloe vera* was also done by using DPPH method and found that *Aloe vera* is also a natural antioxidant.

Key words: *Aloe vera*, Aloin, HPTLC, Antioxidant activity

Introduction

The plant *Aloe vera* is universally known and widely cultivated all over India. Therapeutically, *Aloe vera* is used both internally and externally for curing various ailments. *Aloe vera* has long been used as a remedy in many cultures. As the "wonder plant" has multiple uses from being an antiseptic, anti-inflammatory agent, helps in relieving cancer and diabetes, being in cosmetic field (Davis RH, 1989). *Aloe vera* is undoubtedly, the nature's gift to humanity for cosmetic, burn and medicinal application and it remains for us to introduce it to ourselves and thank to the nature for its existence as never-ending gift. Aloe preparations including products based on both the gel and the leaf, are used among other reasons as laxatives, in creams for skin, in functional foods, and as treatment for a wide range of diseases (V. Steenkamp,2007). The gel found in the leaves is used for soothing minor burns, wounds, and various skin conditions like eczema and ringworm. The extracted Aloe juice is used internally to treat a variety of digestive condition (Kokate C K). There are more than 200 different chemical constituents found in *Aloe vera* namely amino acids, Anthraquinone, Lignin, Mono-and polysaccharides, Saponins, sterols, Vitamins, Salicylic acid, Minerals, Enzymes, in which Aloin is reported as a main chemical constituent (M. Ahmed, 2013).

Aloin is the main anthraquinone in aloe leaf, which occurs naturally as a mixture of two diastereoisomers, aloin A and aloin B. In addition to these compounds, other compounds including aloenin, aloenin B, and isoaloesin have been related to the biological properties of *Aloe vera* extracts (S. P. Joshi, 1997). *Aloe vera* also contains several potentially bioactive compounds including salicylates, magnesium, lactate, (Joshi SP, 1997). Aloe is widely used in the cosmetic, pharmaceutical and food industries because it contains antioxidants which may increase the shelf life and nutritional value of food.

HPTLC is a sophisticated and automated form of TLC. *HPTLC* is an invaluable quality assessment tools for the evaluation of active biomarker. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally numerous samples can be run in a simple analysis there by reducing analytical time with HPTLC. The analysis can be carried out using different wave lengths of light - 254nm. short wave UV light, 366nm long-wave UV light, and 302nm mid wave

light. High-performance thin layer chromatography (HPTLC) is the usual technique for the determination of individual components in *Aloe vera* leaf extracts. There are already several examples of the use of HPTLC for the analysis of aloin derivatives (Pandey, D. K., 2012) The objective of the present work was to develop an accurate, specific and reproducible method for the estimation of Aloin from *Aloe vera* and its antioxidant activity.

Material and Methods

Sample preparation

Aloe vera leaves were collected from the herbal garden of The Himalaya Drug Company, Dehradun. These were washed with water and removed the rinds. The inner gel scrapped, cut into pieces and kept dried on petridish. The dry gel particles were scratched and weighed, then dissolved in methanol (250mg in 50ml). The resulting solution was filtered. This solution was used for HPTLC analysis.

Standard solutions

Dissolved 10mg of reference compound of Alon in 10 ml of methanol. 0.1 µL of the solution contains 100mg aloin.

Chromatographic Conditions

The HPTLC system was composed of a CAMAG Linomat-5 sample applicator and CAMAG TLC scanner-3 provided with CATS software. The stationary phase was composed of pre-coated silica gel 60F254 TLC plates (20×10). Samples were administered to the plate via Linomat-5 with a 100ul Hamilton syringe. The twin through development chamber was saturated with ethyl acetate: Methanol: water (100:13.5:10) as mobile phase (Wagner and Bladt, 1996). Applied different volume of samples and standard band wise with CAMAG Linomat 5, distance from lower edge, 8mm and distance from left edge, 20mm. Evaluation was done by using CAMAG TLC Scanner and CATS evaluation software; scanning by absorbance at 350nm. Evaluation via peak area.

Antioxidant assay

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of (Brand-Williams et al 1995) with modifications.

The following concentrations of extracts were prepared 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL, 50µg/mL, 60µg/mL and 70µg/mL. A stock solution of the sample (50mg/ml) was diluted for 7 concentrations. Each concentration was tested in duplicate. The portion of sample solution (100µl) was mixed with 300µl of 0.2mM 1, 1-Diphenyl-2-2picrylhydrazyl (DPPH, in 96% distilled ethanol) and allowed to stand at room temperature for 30 minute under light protection. The absorbance was measured at 517nm.The scavenging activity of the samples at corresponding intensity of quenching DPPH. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Inhibition % = Ac-As/Ac×100

Where Ac is the absorbance of the control As is the absorbance of the sample

Results and Discussion

The Rf values and color of the resolved bands were noted after development of the silica plate upto the height of 8.5cm. The bands subjecting to aloin were found to be yellow in color under 366nm. The Rf was found to be 0.36. The densitometric scanning at 350nm of aloin and A*loe vera* are in fig-1 and fig-2 respectively. The spectra generated for aloin and methanol extract of *Aloe vera* leaves at wavelength range of 200-400 nm also matches as given in Fig-3, which indicates the presence of aloin in the sample.

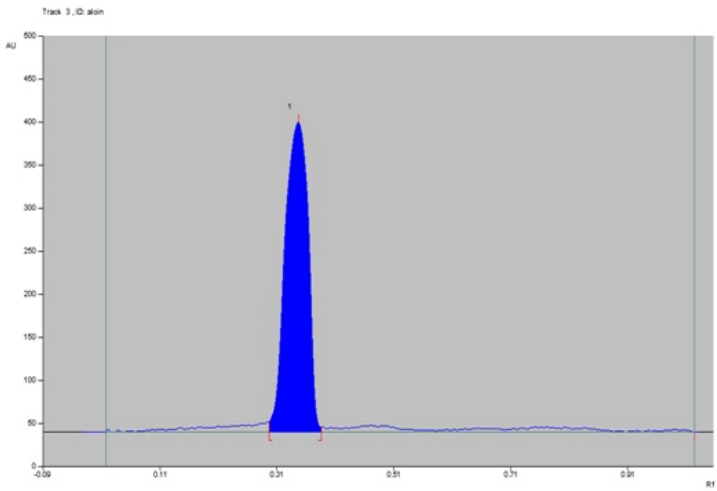

Figure-1 HPTLC Chromatogram of standerd aloin at Rf 0.36 at 350nm

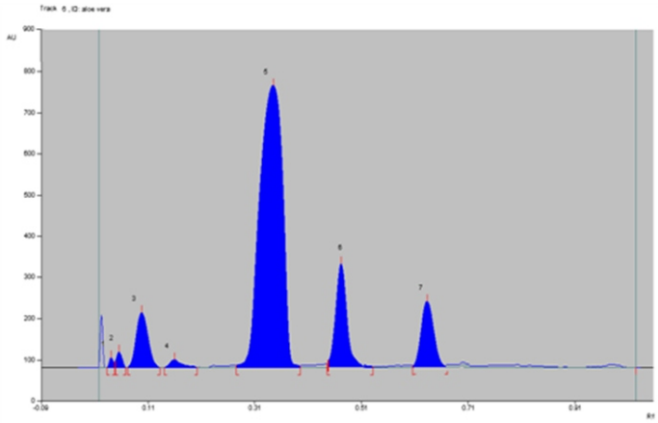

Figure-2 HPTLC Chromatogram of aloe vera at 350nm

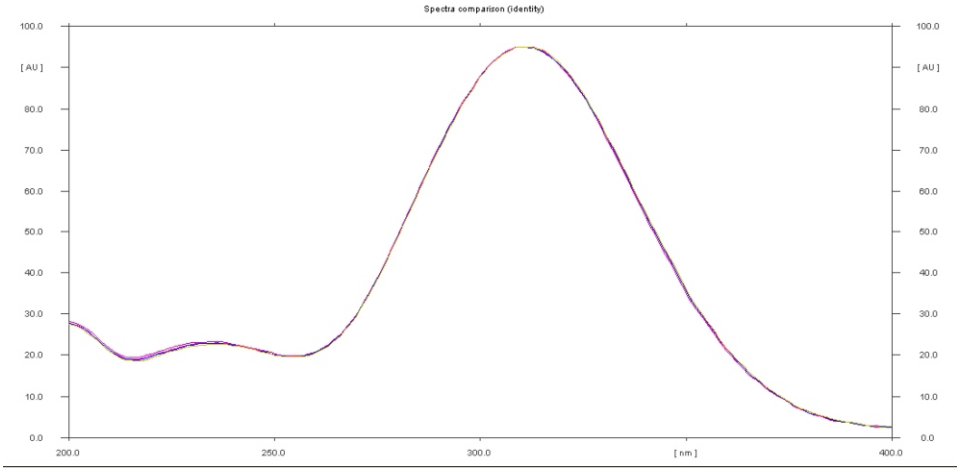

Figure-3 Overlaying of absorption spectra of methanol extract of *Aloe vera* with standard aloin.

The selected solvent system gave well defined peak resolution. With the help of the peaks and analyzing the UV spectra, the band at Rf 0.36 was idenfified as aloin. The TLC plates were visualized at 254nm and 366nm in camag visulizing cabinet. Better visulization was obtained at 366nm because of the florescence of aloin. Linear calibration curve was found between 200ng-1000ng of aloin. The correlation coefficient for a calibration curve was founf to be 0.99957 for aloin.

The regression equation for the calibration plot for aloin is Y=283.238+2.183X. percentage of aloin was determined by using the peak area parameter. This HPTLC method is specific for aloin because it resolved the compound (Rf=0.36) well in the presence of other components in *Aloe vera*. The aloin content was found 2.43%w/w. The method allows reliable quantification of aloin and good resolution from other constituents of *Aloe vera*.

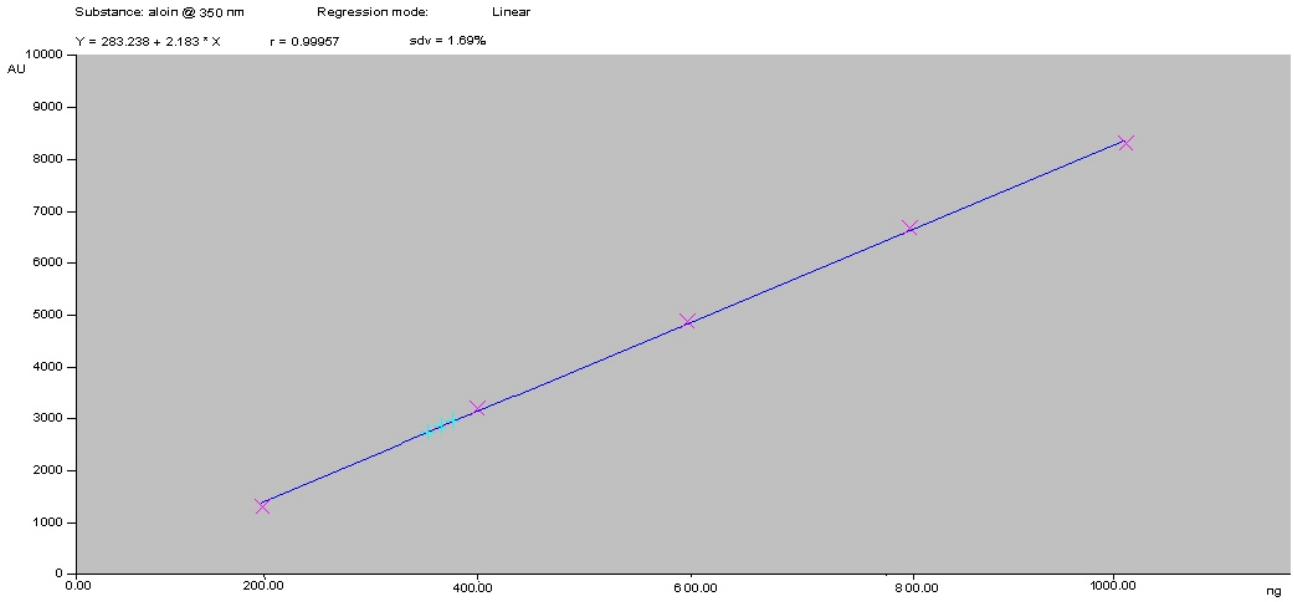

Figure-4 Linear calibration curve of aloin (200ng-1000ng)

Antioxidant activity of *Aloe vera* leaves

Our study emphasizes the antioxidant potential of *Aloe vera* methanolic leaf extracts. Methanolic extract induced the best extraction yield and more complex composition of phenolics (Pop RM 2013). The methanol extracts of *A.vera* were also screened previously for their in vitro antioxidant activity (Lopez A 2013). Biological activities of *A. vera* may be due to the synergistic action of these compounds, rather than from a single defined component [46].

The antioxidant activity of methanolic extract of A*loe vera* leaves is determined by the free radical DPPH reduction method, Fig-5. DPPH radical scavenging method is an extensive procedure to evaluate the free radical scavenging ability of various samples (Brand-williams W,1995). The effect of antioxidants on DPPH radical scavenging was supposed to be due to their hydrogen donating ability.

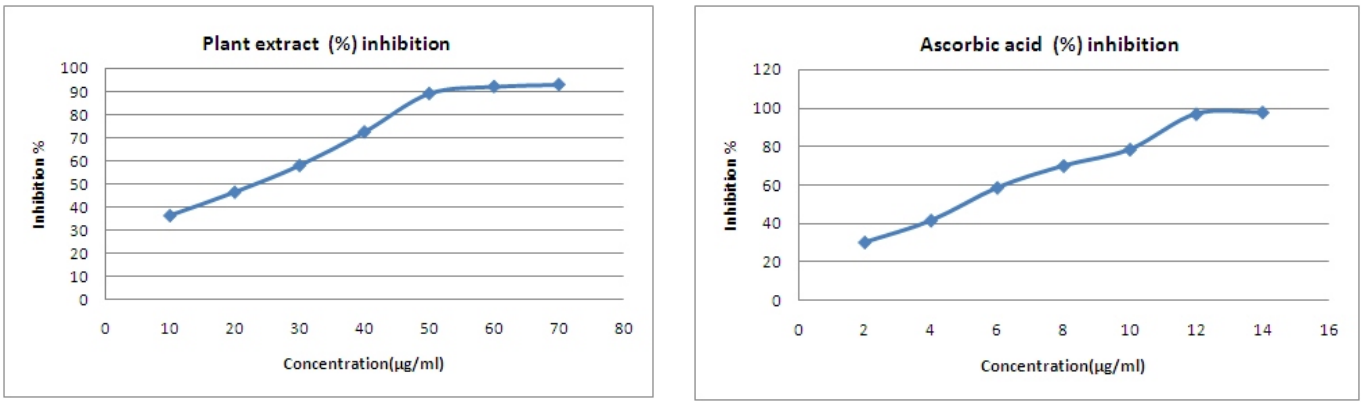

Figure-5 DPPH radical scavenging activity of aleo vera plant extract and ascorbic acid

The antioxidant activity has been given from the IC_{50}. It is the concentration of the antioxidant giving 50% inhibition of DPPH in the test solution. The evaluation of the antioxidant activity of the methanolic extract of *aloe vera* and ascorbic acid show that the IC_{50} values are 27.92µg/mL and 5.19µg/mL respectively. Scanning of *aloe vera* plate at 350nm shows the presence of more than seven constituents present in it as shown in Fig-2. The antioxidant activity of *A. vera* may be due to the synergistic action of these compounds, or may be due to the active constituent aloin.As reported earliar the aloin is known as antioxidant, antimicrobial, anticancer, anti-inflammatory, (S.kumar 2017).

Conclusion

Among the Complex mixtures of biologically active compounds in the leaves of *Aloe vera*, aloin can be used as an analytical marker to determine the quality of plant material. The aloin content was found 2.43%w/w. The active ingredients hidden in its leaves have the power to soothe human life and health in many ways. The present study showed that *A. vera* is a promising source of bioactive compounds which has a good antioxidant activity. The in vitro antioxidant activity showed that the *Aloe vera* has a good antioxidant activity. Good antioxidant properties of the *Aloe vera* could be considered for applications in food, medicine and cosmetic industries.

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Antimicrobial Activity of Plant Essential oils and Their Emerging Role in Food Sector

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DOI 10.51129/ujpah-2020-29-2(10)

Abstract-Food preservatives are used to increase the shelf life of food and to maintain the quality for longer time. Natural methods of preservation usually aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive[25]. In the last scenario, no herbal Ayurvedic preservative had been considered with respect to the use of chemical preservative. Increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils having antimicrobial properties.

Essential oils from medicinal plants are potential source of novel antimicrobial compounds especially against food spoilage pathogens. The aim of this project was to compare the antimicrobial activity of essential oils collected from Lemongrass (*Cymbopoga ncitratus)*, Clove *(Syzygiumaromaticum*) and Tulsi (*Ocimum sp*.) against food spoilage organisms.

In this study the essential oil of Lemongrass, Clove and Tulsi were investigated for its activity against Gram negative bacteria *Escherichia coli*, Gram positive bacteria *Micrococcus luteus, Staphylococcus aureus* and *Bacillus cereus, yeast Candida albicans* and fungus *Aspergillus niger, Chaetomium globosum* and *Penicillium funiculosum* using agar well diffusion method. The antimicrobial activity was evaluated by measuring the zone of inhibition. The oils at 30% concentration completely/partially inhibited the growth of food spoilage pathogens. The strongest inhibition activity was observed in Lemongrass oil against all the test organisms; thus it was found more effective as compared to Clove oil and Tulsi oil.

The successful effectiveness of Lemongrass oil could also play a major role in replacing the chemical preservative.

Keywords: *Ocimum, Teniflorum,* Lemon grass and *Cymbopogon citratus*

Introduction

Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens[1].

The use of natural antibiotics agents are the best alternative to synthetic or chemical antibiotics. It prevents development of antimicrobial resistance in bacteria and fungus and also is devoid of side defects. The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against currently available antibiotics. However, traditional plants have been proved to be better sources for novel antimicrobial drugs. Growing demands for more natural and preservative-free cosmetics promoted an idea of the replacement of synthetic preservatives with essential oils (EOs) of antimicrobial properties. The antimicrobial effect of essential oil depends on content, concentration and interactions between the main active compounds. Effective preservatives should be characterized by a

broad spectrum of antimicrobial activity at a minimum concentration. Down the ages, numerous essential oils extracted from plant materials have been used for their aroma, flavor, bactericidal, preservative and medicinal properties[2].

Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine[3,4]. Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savoury and fennel have been successfully used either alone or in combination with other preservation methods[5].

Ocimum tenuiflorum, also known as *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities [5]. It has been one of the most valued and holistic herbs used over years in traditional medicine in India and almost every part of the plant has been found to possess therapeutic properties[5].

There are 54 volatile components present in Tulsi leaves. The most abundant of the are monoterpenes and sesquiterpenes; in particular monoterpenes such as camphor, cineole, estragol, and eugenol, followed by sesquiterpenes, such as germacrene, caryophyllene, bisabolene. Traditionally, Tulsi is used in different forms; aqueous extracts from the leaves (fresh or dried as powder) are used in herbal teas or mixed with other herbs or honey to enhance the medicinal value. Traditional uses of Tulsi aqueous extracts include the treatment of different types of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart disease[6]. Oils extracted from the leaves and inflorescence of Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, and antipyretics; stress

Figure-1 Leaf of *Ocimum sanctum* and *Ocimum sanctum oil*

reducers and inflammation relievers; and as anti-asthmatic, hypoglycemic, hepatoprotective,hypotensive, hypolipidemic, and immunomodulatory agents[5]. Antioxidants present in Tulsi are significant in the prevention of human illness as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quencher of singlet oxygen formation. Free radicals possess the ability to reduce the oxidative damage associated with many diseases including neurodegenerative diseases, cancer, cardiovascular diseases, cataract and AIDS[7-9]. Antioxidants like Tulsi through their scavenging power are useful for the management of these diseases.

Clove (*Syzygium aromaticum*) on the other hand, is a plant widely cultivated in Spice Islands, Indonesia, Pemba and Zanzibar, though earlier production of the plant was in China.

Figure-2 Leaf of Cloveand Clove oil

This essential oil comprises in total 23 identified constituents, among them eugenol (76.8%), followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenylacetate (1.2%) as the main components. Clove oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and is used traditionally as a savouring agent and antimicrobial material in food[10-12]. In addition, clove oil is used as an antiseptic in oral infections[13,14]. This essential oil has been reported to inhibit the growth of moulds, yeasts and bacteria[15]. It was effective against *L. monocytogenes* and *S. Enteritidis* in Tryptone Soya Broth and cheese[16]. The high levels of eugenol contained in clove essential oil are responsible for its strong biological and antimicrobial activities. It is well known that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast[17,18]. It is used in the seasoning of food.

Cymbopogon citrates commonly known as lemongrass is an herb which belongs to the grass family of *Poaceae*. It is utilized for its distinct lemon flavour and citrusy aroma. It is a tall, perennial grass which is native to India and tropical regions of Asia. It is a coarse and tufted plant with linear leaves that grows in thick bunches, emerging from a strong base and standing for about 3 meters in height with a meter-wide stretch.

The genus *Cymbopogon* comprises of 55 species of grasses, two of which are referred to as lemongrass. These are *Cymbopogon citratus,* which is famously preferred for culinary use and *Cymbopogon flexuosus,* which is used in the manufacturing of fragrances because of its extended shelf life, owing to the low amount of myrcene in that variety.

Lemongrass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavour. Lemongrass oil used as a pesticide and preservative, is put on the ancient palm-leaf manuscripts found in India as a preservative.

One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*)is citral (3,7-dimethyl-2,6-octadien-1-al). Lemongrass oil has been found to contain up to 75-85% citral. Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral), beta-myrcene (MYR, 7-methyl-3-methylene-1,6 octadiene), limonene, piperitone, citronellal, carene-2, alpha-terpineole, pinene, farnesol, proximadiol, and (+)-cymbodiacetal. The volatile oil from the roots contains 56.67% longifolene-(V4) and 20.03% selina-6-en-4-ol.

Figore -3 Leaf of lemongrass and lemongrass oil

In particular, a study of *Cymbopogon martinii* isolated fatty acids, common sterols, and 16-hydroxypentacos-14(z)-enoic acid*Cymbopogon citratus* has been cultivated over many years for medicinal purposes in different countries throughout the world. Lemongrass is an aromatic storehouse of essential nutrients providing an array of health benefits. It is a source of essential vitamins such as vitamin A, vitamin B1 (thiamine), vitamin,B2 (riboflavin), vitamin B3(niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folate, and vitamin C. It also provides essential minerals such as potassium, calcium, magnesium, phosphorous, manganese, copper, zinc and iron, which are required for the healthy functioning of the human body. It offers no harmful cholesterol or fats. The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties[19]. It can be used in cleaning wounds and treatment of skin diseases such as ringworm. It can also be used in food poisoning, staphylococcal infections, and other common infections.

The oil has been found to possess bactericidal and anti-fungal properties, which is comparable to penicillin in its effectiveness[20]. As a bactericidal agent, the lemongrass oil was found to be effective against many bacterial species including *Acinetobacter baumanii*, *Aeromonas veronii*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella enterica*, *Serratia marcesens*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Corynebacterium equii*, *Staphylococcus aureus* and so on[21-24].

Antimicrobial activity of the *Cymbopogon citratus* (lemongrass) essential oil against food-borne pathogens was determined to investigate its potential for reducing microbial population of food products. Previous reports suggest that lemongrass essential oil is a safe natural flavour complex, preservative, and food spoilage inhibitor capable of reducing the risk of diseases associated with contaminated products.

The aim of our study was to gain the attention on usage of essential oils as antimicrobial agents and we use lemongrass oil against a wide spectrum of food spoilage pathogenic two strain of Gram negative bacteria namely; *Escherichia coli,* and three strain of Gram positive bacteria namely; *Micrococcus luteus, Bacillus cereus, Staphylococcus aureus, and against some fungus namely Candida albicans, Aspergillus niger Penicillium funiculosum and Chaetomium globosum.*

Material and Methods

Lemongrass oil, Clove oil and *Ocimum* oil was investigated for activity against various selected microorganisms. The material and methods needed for this study are listed below in accordance to their source of availability and grades.

Media and Chemicals

- Nutrient Agar(NA)
- Mueller Hinton Agar (MHA)
- Chloramphenicol Yeast Glucose Agar (CYGA)
- Antibiotic Assay Medium 11
- Antibiotic Assay Medium 19
- Sodium Chloride
- Tween-80

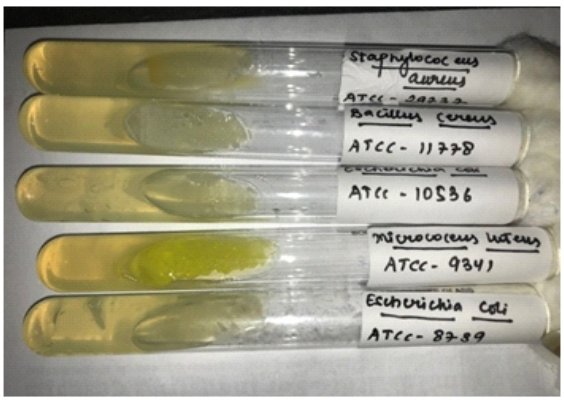
Procurement of lemongrass oil

The essential oil of lemongrass was procured from SIGMA LRICH, India.

Fungal and Bacterial strains

The test organisms used in this study was taken from Department of Microbiology of Shriram Institute for Industrial Research New Delhi as a standard strain and the following details are given below.

Bacterial strain	ATCC No.
Micrococcus luteus	9341
Escherichia coli	8739
Escherichia coli	10536
Staphylococcus aureus	29737
Bacillus cereus	11778



Fungal strain	ATCC No.
Aspergillus niger	16404
Candida albicans	3471
Chaetomium globosum	6205
Penicillium funiculosum	11797

Figure- 4 Bacterial cultures used in study

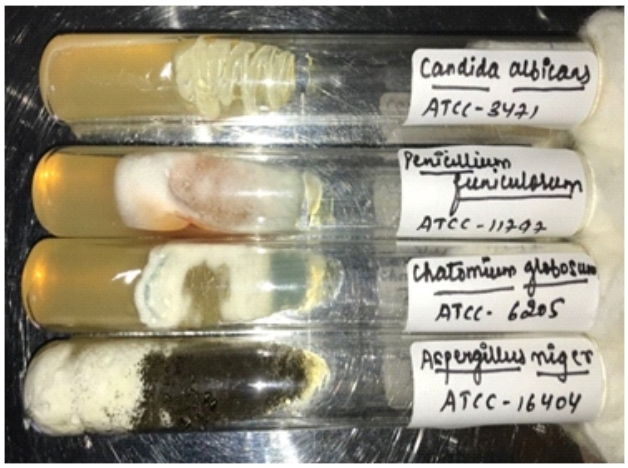


Figure - 5 Fungal cultures used in study

Propagation and maintenance of test organisms

The bacterial test organisms were streaked on the Nutrient Agar slants and were incubated overnight at 37˚C and fungus test organisms were streaked on the Chloramphenicol Yeast Glucose Agar slants and were incubated for 5 days at 22˚C.

Preparation of concentrations of oil

The 30% concentrations (v/v) ofoil were prepared aseptically in sterile tween-80.

Antimicrobial activity

The testing of the bacterial and fungal cultures for the inhibitory effect of essential oil was done at 30% concentrationby using agar well diffusion method.

Agar Well Diffusion Assay (Zone of Inhibition Evaluation): Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 0.5 McFarland density of bacterial and fungal culture was adjusted using normal saline (0.85% NaCl) using densitometer to get bacterial and fungal population of 1.0 x 10^8 cfu/ml. 100µl of each

of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA (Meuller-Hinton agar), mixed well and poured into sterile Petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 100 µl of respective essential oil were pipette out into the well in assay plates. Bacterial plates were incubated overnight at 37°C and fungal plates were incubated for 5 days at 22°C. Following incubation, petri-plates were observed for the inhibition zones, diameters of which were measured by using Vernier Callipers.

Results and Discussion

Lemongrass oil at concentration of 30% was found the most effective essential oil against all the selected microorganisms as compared to Clove oil and Ocimum oil. All the selected microorganisms showed difference in their sensitivity against three different essential oils. The strongest inhibition activity was observed in Lemongrass essential oil against *Bacillus cereus* and *Candida albicans*. Results of antimicrobial activity of essential oils using agar well diffusion method revealed that Lemongrass is much effective and broad spectrum of antimicrobial activities as compared to Clove oil and Tulsi oil against food spoilage organisms.

Our observation revealed that only one strain of bacteria was resistant against Lemongrass while remaining all microorganisms were sensitive.

Table -1 Antimicrobial activity of lemongrass oil against various selected microorganisms-

S. No.	Name of microorganisms	ATCC No.	Zone of inhibition (30%)		Average
1.	*Escherichia coli*	8739	15.80 13.48	12.80 13.45	13.88
2.	*Escherichia coli*	10536	NZO	NZO	0.0
3.	*Micrococcus luteus*	9341	18.51 21.98	21.09 20.30	20.47
4.	*Bacillus cereus*	11778	20.21 18.99	18.16 20.29	19.41
5.	*Staphylococcus aureus*	29737	14.08 15.90	27.03 21.22	19.56
6.	*Aspergillus niger*	16404	50.08 51.06	50.36 49.08	50.15
7.	*Candida albicans*	3471	35.63 35.38	39.63 38.08	37.17
8.	*Chaetomium globosum*	6205	36.21 35.27	33.84 33.65	34.73
9.	*Penicillium funiculosum*	11797	15.47 13.89	16.32 14.98	15.17

*NZO No zone observed

*Zone of inhibition in mm. Diameter including well diameter of 6.0mm.

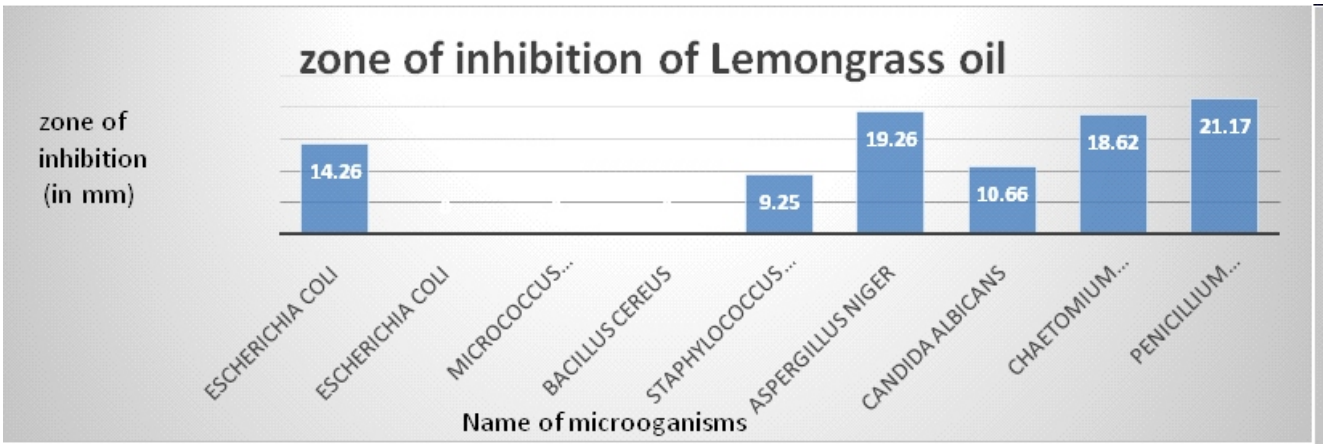


Figure -6 Zone of Inhibition of Lemon Grass Oil

Table-2 Antimicrobial activity of clove oil against various selected microorganisms-

S. No.	Name of Microorganisms	ATCC No.	Zone of inhibition (30%)		Average
1.	*Escherichia coli*	8739	11.31 10.37	11.00 11.58	11.07
2.	*Escherichia coli*	10536	11.24 11.00	13.65 15.31	12.8
3.	*Micrococcus luteus*	9341	17.75 19.19	9.87 11.40	14.55
4.	*Bacillus cereus*	11778	15.07 14.23	13.89 12.23	13.86
5.	*Staphylococc us aureus*	29737	9.27 8.82	10.95 10.04	9.77
6.	*Aspergillus niger*	16404	34.43 43.23	46.32 42.25	41.56
7.	*Candida albicans*	3471	17.79 19.92	16.90 17.93	18.16
8.	*Chaetomium globosum*	6205	40.28 40.56	37.01 40.30	39.53
9.	*Penicillium funiculosum*	11797	35.18 37.50	45.59 43.10	40.34

*NZO No zone observed
*Zone of inhibition in mm. Diameter including well diameter of 6.0mm.

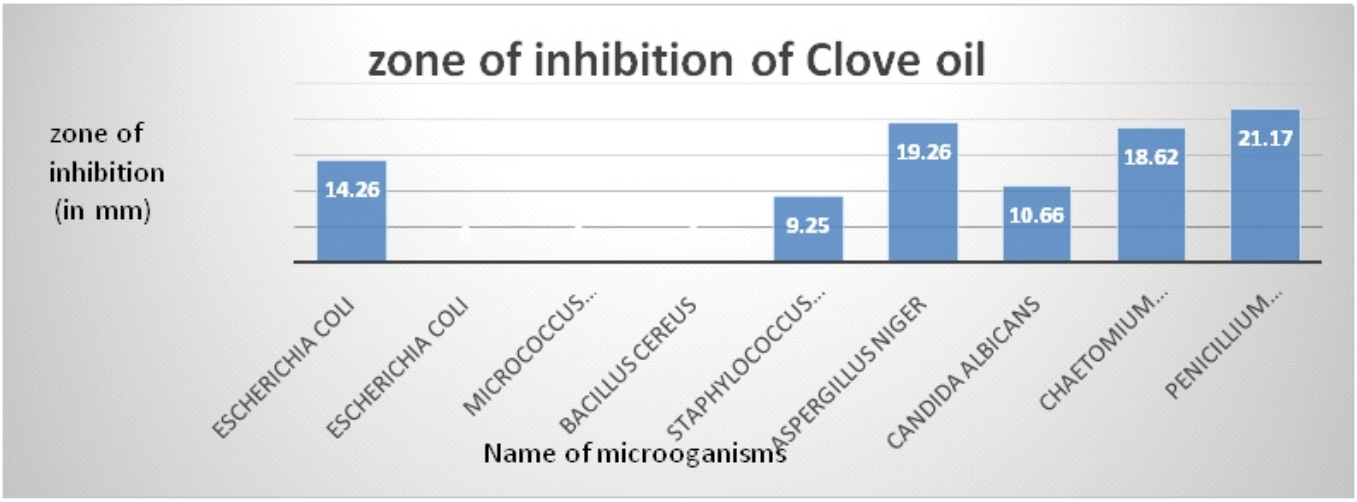


Figure-7 Zone of inhibition of Clove Oil

Table-3 Antimicrobial activity of *Ocimum* oil against various selected microorganisms-

S.No.	Name of microorganisms	ATCC No.	Zone of inhibition (30%)		Average
1.	*Escherichia coli*	8739	15.38 13.95	13.42 14.29	14.26
2.	*Escherichia coli*	10536	NZO	NZO	0.0
3.	*Micrococcus luteus*	9341	NZO	NZO	0.0
4.	*Bacillus cereus*	11778	NZO	NZO	0.0
5.	*Staphylococc us aureus*	29737	10.12 9.24	9.12 8.50	9.25
6.	*Aspergillus niger*	16404	19.79 19.54	20.98 16.76	19.26
7.	*Candida albicans*	3471	9.85 10.44	11.47 10.86	10.66
8.	*Chaetomium globosum*	6205	18.59 18.27	19.13 18.47	18.62
9.	*Penicillium funiculosum*	11797	20.84 19.41	21.84 22.58	21.17

*NZO No zone observed
*Zone of inhibition in mm. Diameter including well diameter of 6.0mm.

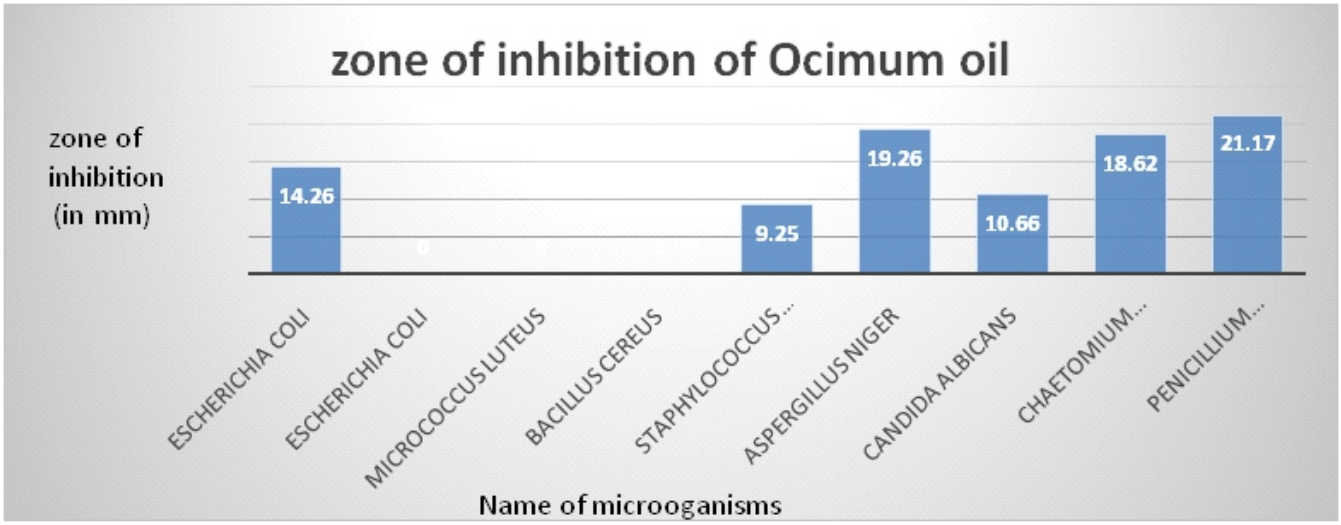


Figure-8 Zone of inhibition of Ocimum o

Conclusion

The major concern is extensive uses of chemical food preservatives. The present scenario shows overuse of chemical preservatives in food products, this may not only lose the natural property of food but also effect the consumer's health.

Increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils of antimicrobial properties

The comparative effects of essential oils on the various test organisms are demonstrable indications of the oil as an antimicrobial agent. Thus a study had been carried out to show that an herbal product Lemongrass oil is much potential against food spoilage organisms as compared to Clove and Tulsi oil.

The results conclude that Lemongrass Oil, Clove Oil and Tulsi Oil possesses inhibitory activity in the series Lemongrass Oil > Clove Oil >Tulsi

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Evaluation of Toxicity and Antidiabetic Activity of Ethanolic Extract of Flowers of *Moringa Oleifera* Against Dexamethasone Induced Hyperglycemia in Albino Wistar Rats

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DOI 10.51129/ujpah-2020-29-2(11)

Abstract-Diabetes is a defect in the ability of the body to convert glucose (sugar) to energy. Glucose is the main source of energy in our body. When food is digested it is metabolized into fats, proteins, or carbohydrates. Glucose is then transferred to the blood and is used by the cells for energy production. To investigate the antidiabetic effect ethanolic extracts of flowers of *Moringa oleifera* against dexamethasone induced insulin resistance in wistar albino rats. To study the antidiabetic effect, flowers of *Moringa oleifera* were collected and authenticated, extracted and investigated for acute toxicity and dexamethasone induced hyperglcemia. The animals treated with EEMOF at a dose of 100mg/kg and 200mg/kg prevented the development of hyperglycemia, hypercholesteremia and hypertriglyceridemia in dexamethasone induced insulin resistance models. Oral administration of *Moringa Oleifera* 100mg/kg and 200mg/kg reduces serum glucose, triglyceride, total cholesterols and LDL concentration and improve the concentration of HDL in dexamethasone administered rats. The lignin *Moringa Oleifera* showed significant anti-diabetic effect in rats after oral administration. The present study demonstrated that *Moringa Oleifera* could be useful in Management of diabetes associated with abnormalities in lipid profiles. Further study need to isolate, identify the active compounds and find out the possible mechanism of actions.

Keywords: *Moringa oleifera,* Dexamethasone, Hyperglycemia, Ethanolic Extract

Introduction

Moringa Oleifera is the most cultivated species of the genus *Moringa*, the only genus in the Moringaceae plant family. Common names include moringa, wand tree (long and thin triangular pods), horseradish (from the taste of roots, Reminiscent of horseradish) and Ben oil tree or benzoyl tree (from the oil that comes from the seeds)[6]. *M. Oleifera* is a fast-growing, drought-resistant tree native to the tropical and subtropical regions of southern Asia. It is widely cultivated for its young pods[7] and leaves used as vegetables and for traditional herbal medicine. It is also used for water purification. *M. Oleifera* is considered an invasive species[8]. t shows Pharmacognostical and Preliminary Phytochemical Studies[9], Adaptogenic Activity[10], anthelmintic activity[11], *in-vitro* antibacterial activity[12], Adaptogenic Activity[13] in Anti-Obesity Activity [14] etc.

Diabetes is a defect in the ability of the body to convert glucose (sugar) to energy. Glucose is the main source of energy in our body. When food is digested it is metabolized into fats, proteins, or carbohydrates. Glucose is then transferred to the blood and is used by the cells for energy production. For transferring of glucose, the hormone - insulin is needed which is mainly secreted by pancreatic beta cells. Diabetics mellitus is a type of metabolic disorder that is characterized by increased glucose production in the blood with disturbances in metabolism of carbohydrate, protein and fat mainly due to defects in insulin secretion, its action or both[1].

If this hyperglycemic stage of diabetes [2,3,4] persists for a long time, it is associated with long-term complications like improper functioning and failure of different organs causing deep damage to the eyes, kidneys, nerves, heart, and blood vessels [5].

Dexamethasone is an effective and greatly selective glucocorticoids used in the treatment of inflammation. Large exposure of glucocorticoids impairs insulin sensitivity, leads to the generation of metabolic syndrome as well as insulin resistance and hypertension. The mechanism which

dexamethasone induces peripheral insulin resistance is in inhibiting GLUT-4 translocation, and rising lipase activity in adipose tissue leads to cause impairment of Endothelium-dependent vasodilatation[15]. Dexamethasone increases the triglycerides levels causing an difference in lipid metabolism leads to hyperlipidemia and increases glucose levels causes to hyperglycemia Pharmacological doses of glucocorticoids induces ob gene expression in rat adipose tissue within 24 hrs and is followed by a complex metabolic changes ensuing in decrease in food consumption causing reduction in body weight and also occurred with by diabetes and generation of Insulin resistance with improved glucose and triglycerides levels. In this experiment administration of dexamethasone for 10 days resulted in improved glucose, triglycerides, cholesterol, insulin, levels[16].

Material and Methods

Plant Material

Plant material flower of *Moringa oleifera* was purchased from local market of Indore (M.P.).

Authentication: *Moringa oleifera* was authenticated at Department of Botany, Govt. Holkar Science College, Indore (M.P.).

Experimental Animals

Albino Wistar rats of both sexes were used in approximately the same age group after acclimatization for a week under laboratory conditions. They received a standard diet for rodent pellets (Lipton India) and *ad libitum* water. The animals had free access to food and water and were kept under a 12.12 h light and darkness cycle. All experiments were performed during the day from 9:00 am to 5:00 pm. The protocol was approved by the institutional committee on animal ethics and animal care was carried out in accordance with the guidelines of the committee for control and supervision in animal experiments (CPCSEA), representative of Animal Welfare, Government of India (Protocol No. SVCE/BIO/2019/004).

Instruments

UV-Visible Spectrophotometer (UV-1800 Shimadzu, Model, Mfg by Shimadzu Corporation), Centrifuge (Research centrifuge, Mfg by Remi Instruments Ltd, Mumbai), Tissue Homogenizer (Type: RO-127A Elect, IND.Ltd, Remi Instruments Division), Sonicator (Pci made in Mumbai) Milli pore water collector (Mfg by TKA smart pure Made in Germany), Soxhlet apparatus (Agarwal) Rotory evaporator (Medika instrument Mfg co.) UV chamber (Singhla sciences, Ambala).

Chemicals Used In this Study

Dexamethasone, glipinclamide, Heparin sodium injection I.P, Sodium lauryalsulphate L.R), Acetic acid(L.R), Thiobarbicturic acid (L.R), Tri chloro acetic acid (L.R), Phosphate buffer (K_2HPO_4) (L.R), 5,51-dithiobis(2-nitrobenzoic acid) 99% extra pure(L.R), Sulphosalicylic acid (L.R), Sodiumpyro Phosphate di basic (L.R), PhenazineMethosulphate (R & D), Nitro blue tetrazolium (L.R), Coomassie brilliant blue (Bradford Reagent) (R & D), Ethanol absolute (L.R), Sodium Phosphate dibasic (L.R),

Extraction and Identification of Phytoconstituents

Extraction of plant material

The air dried flower of *Moringa Oleifera* 500gms each were coarse powdered and extracted with ethyl alcohol. The crude extract was further filtered and evaporated by the aid of rotary evaporator. The final mass is weighed and preserved for further use[17,18].

Preliminary phytochemical screening

Preliminary phytoconstituents present in the hydroethanolic extract of *Moringa oleifera* plant were identified based on the chemical tests described by Kokate *et al.,* 1994[19].

***In-vivo* studies**

Acute oral toxicity study (OECD 423)

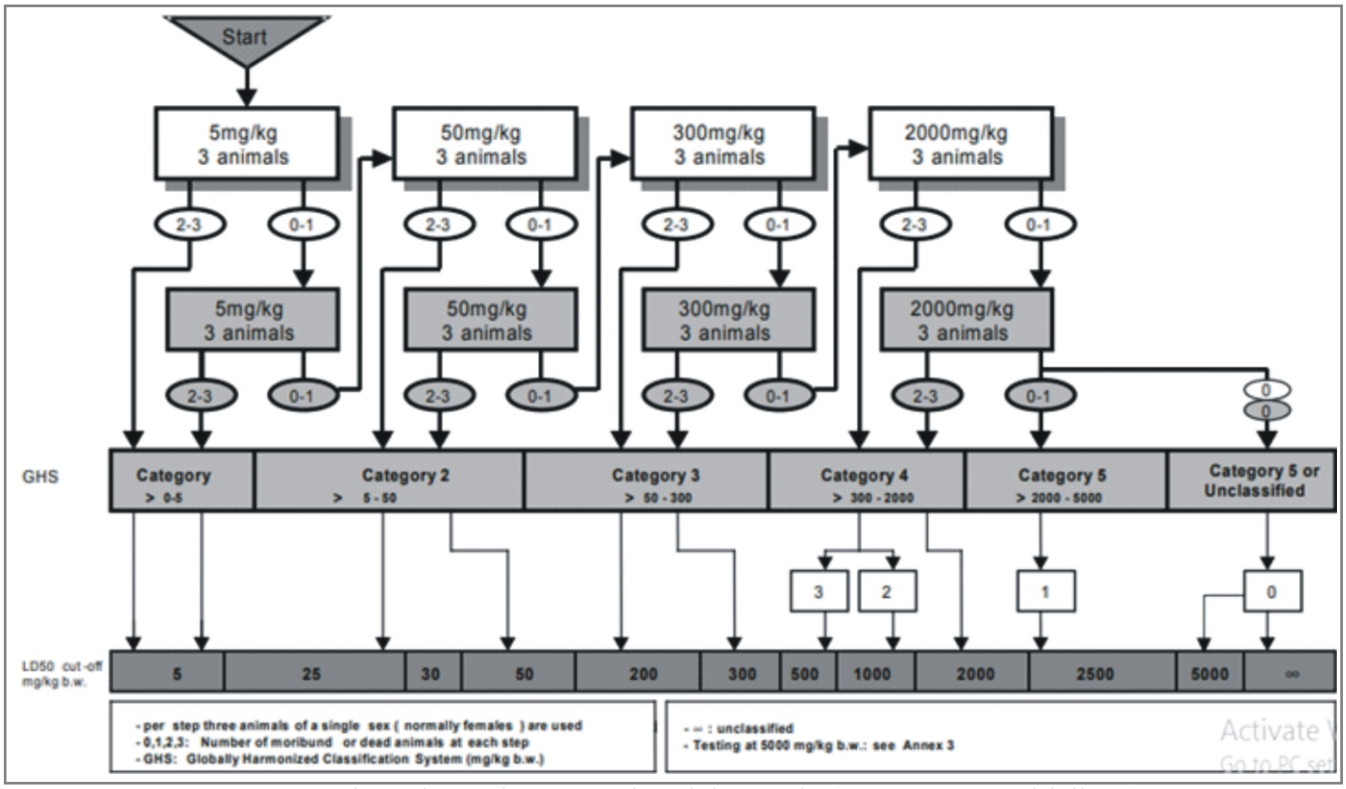

Figure-1 Flow chart of acute oral toxicity study (OECD 423 Guidelines)

To carry out the oral toxicity study, the OECD guidelines 423 were followed: it is a gradual procedure with three animals of one sex for each step. Depending on the mortality and/or 5, 50, 300, 2000 mg/kg body weight and the results allow classifying a substance as animal morbidity, some steps may be needed to judge the toxicity of the test substance. This procedure has an advantage over other methods due to the minimal use of animals and allows acceptable data. The method uses defined and classified doses according to the globally harmonized system. The initial dose for the ethanol extract was 2000 mg/kg of body weight (p.o). The dose was administered to rats that were fasting overnight with *ad libitum* water and observed for signs of toxicity. The same dose was tested once again with three other rats and was observed for 72 hours to detect symptoms such as change in skin color, salivation, diarrhea, sleep, tremors, convulsions and even respiratory, autonomous and CNS effects[20].

Sub acute toxicity study (OECD 407)

To carry out the subacute oral toxicity study, OECD 407 guidelines - A 28-day oral toxicity study was performed with repeated doses in rodents. The duration of the study was 28 days. The rat was given at a dose of 200 mg / kg. Each group is composed of ten animals (five animals / sex / group). The drug was administered orally once a day for 28 days. On the 29th the animals were anesthetized and the blood was collected by a retro-orbital puncture. The hematological parameters were evaluated. Serum was separated and biochemical parameters were estimated. The animals were sacrificed and the organs were removed and weighed. The organs were maintained in 10% formalin and used for histopathological analysis[21].

Hematological studies

The following hematological parameters were estimated by standard procedures.

Blood samples were drawn by cardiac puncture and haematological parameters were analyzed by autoanalyzer[22].

I. Total R.B.C. count
ii. Total W.B.C. count
iii. Differential leukocyte count
iv. Haemoglobin (Hb) concentration

Biochemical studies

Blood samples were drawn by cardiac puncture. Blood from three animals was pooled for serum separation. Each serum sample was analyzed by auto analyzer [23].

- Aspartate Aminotransferase (ASAT)
- Alanine Aminotransferase (ALAT)
- Alkaline Phosphatase (ALP)
- Total Bilirubin (TB)
- Total protein.

Experimental Design - Anti Diabetic

Experimental Protocol

30 Rats were divided in to 5 groups (n=6) and the duration of the experiment was 21 days with overnight fasting.

Groups	Treatment
Group-I	Rats received normal distilled water for 21 days.
Group-II	Rats received Dexamethasone 10 mg/kg i.p for a period of 10 days
Group –III	Rats received Dexamethasone 10 mg/kg i.p with low dose of drug 100mg/kg p.o
Group –IV	Rats received Dexamethasone 10 mg/kg i.p with high dose of drug 200mg/kg p.o
Group –V	Rats received Dexamethasone 10 mg/kg i.p along with standard drug Glipenclamide 500mg/kg p.o

At the end of the treatment period, the rats were deprived of food during the night and sacrificed on day 22 and the day under anesthetic with ether and beheaded after recording their final body weight. Blood was taken from each rat for biochemical estimation and the pancreas was quickly isolated, immersed in ice-cold saline and weighed. The pancreas was stored in a freezer (-20 ° C) to estimate the tissue's antioxidant parameters.

Evaluation of Biochemical parameters

Estimation of various biochemical parameters like blood glucose, serum cholesterol, serum triglycerides by using Span diagnostics kit. Estimation of serum HDL-Cholesterol and LDL-Cholesterol was performed by using S.D Kit.

Evaluation of *in-vivo* antioxidant activity

TBARS levels were determined by a modified version of the method described by **Ohkawa *et al*., 1979; Sapakal, 2008.** Glutathione was estimated by the method described by Ellman, 1959. SOD levels in the hearts were determined by the method of McCord and Firdovich method (1969) and modified by Kakkar. Estimation of Catalase by Goth, 1991; Sapakal, 2008.

Results

Percentage yield

The percentage yield of ethanolic extracts of roots of *Moringa Oleifera* was 16%.

Preliminary phytochemical tests

The phytochemical analysis of ethanolic extract of *Moringa Oleifera* revealed the presence of alkaloid, glycoside, sterols, phenols, tannins, flavanoids, terpenes, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, and phytosterols.

Table-1 Preliminary Phytochemical

S. No.	Constituents	Ethanolic Extract
1	Alkaloids	+ve
2	Carbohydrates	-ve
3	Proteins	+ve
4	Phenols	+ve
5	Tannins	+ve
6	Flavonoids	+ve
7	Glycosides	+ve
8	Saponins	+ve

-ve: indicate the absence of compound, **+ve:** indicate the presence of compound

Acute Toxicity studies

Table-2 Acute Toxicity studies of ethanolic extract of the plant *Moringa Oleifera*

Parameters observed	I hr	II hr	III hr	IV hr
Aggressiveness	+	+	+	+
Alertness	-	-	-	-
Alopecia	-	-	-	-
Circling	-	-	-	-
Diarrhoea	-	-	-	-
Edema	-	-	-	-
Eye closure at touch	+	+	+	+
Grip strength	+	+	+	+
Grooming	+	+	+	+
Lacrimation	-	-	-	-
Loss of writing reflex	-	-	-	-
Mortality	-	-	-	-
Nasal sniffing	-	-	-	-
Piloerection	-	-	-	-
Rearing	-	-	-	-
Righting reflex	-	-	-	-
Seizures	-	-	-	-
Straub tail	-	-	-	-
Urine stains	-	-	-	-

4 hours observation in acute toxicity studies of 2000mg/kg of *Moringa Oleifera*

Table-3 Observation in acute toxicity studies at the dose of 2000mg/kg bw p.o dose of *Moringa Oleifera*

Parameters observed	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sub-Acute Toxicity Studies

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into groups of 10 animals (5 male and 5 female). The suspension of ethanolic extract was administered to rats at the dose of 100 & 200 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.

Table-4 Signs of toxicity in sub acute toxicity (28 days)

Parameters observed	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22	Day 24	Day 26	Day 28
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table-5 Change in body weight after the drug treatment *Moringa Oleifera*

Treatment	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	25^{th} day	28^{th} day	% increase
Control	175.83±6.84	179.50±6.28	181.83±6.46	184.83±6.31	187.16±6.01	190.66±6.46	193.66±5.70	10.3637
100mg/kg	177.00±4.43	180.50±4.47	182.83±5.02	186.16±5.40	190.00±6.04	192.50±5.70	195.50±5.63	10.1695
200mg/kg	177.16±8.02	178.83±8.17	182.83±8.23	186.00±7.85	189.00±8.11	192.16±8.43	194.83±8.34	09.6044

Haematological Parameter

Table-6 Haematological Parameter

Haematological parameter	Control	*Moringa Oleifera*	
		100 mg	200mg
Total R.B.C. count ($\times 10^6$ mm−3).	9.09±0.15	8.90±0.12	9.11±0.16
Total W.B.C. Count ($\times 10^3$ mm−3).	12.67±0.22	12.35±0.15	11.23±0.23
Haemoglobin (Hb) (g/dl)	15.61±0.36	14.07±0.30	15.63±0.36
Hematocrit (%).	44.21±1.01	43.61± 1.72	36.4±1.36
Platelets (×103 mm−3).	834.91±24.01	867.21±23.25	739.81±26.86
Lymphocytes(%).	84.7±1.32	81.8±1.33	72.8±1.43
Neutrophils (%).	20.6±0.65	12.6±0.52	19.2±0.91

Data are expressed as mean ± SEM

Table-7 Biochemical Parameters

Biochemical parameter	Control	*Moringa Oleifera*	
		100 mg	200mg
Creatinine (mg/dl)	0.5890±0.079	0.6600 ± 0.049	0.5540 ±0.074
Urea (mg/dl)	15.30 ± 0.47	14.50 ± 0.40	15.20 ±0.57
Triglycerides (mg/dl)	52.20±1.13	51.40±1.08	47.10±1.62
Total Cholesterol (mg/dl)	46.60±1.21	51.40±1.08	54.03±1.67
Total protein (mg/dl)	4.40±0.26	4.20±0.35	3.70±0.26
Albumin (g/dl)	3.20±0.41	3.70±0.33	3.20±0.29
AST (IU/L)	121.41±2.68	121.3±1.65	116.61±2.045
ALT (IU/L)	69.40±1.57	67.60±1.301	68.60±1.108
ALP (IU/L)	112.6±4.67	117.01±0.714	117.41±0.718
T. Bilirubin (mg/dl)	0.2569±0.32	0.267±0.029	0.254±0.023

Dexamethasone induced diabetic model

Blood was collected from each rat for biochemical estimation and pancreas was quickly isolated immersed in ice cold saline and weighed. Pancreas was stored under freezer (-20 °C) for estimation of tissue antioxidant parameters.

Effect of *Moringa oleifera* on serum biochemical parameters

Blood glucose, Total cholesterol, HDL, LDL, VLDL, Triglycerides, levels were estimated in serum. The results were presented.

Serum level of Blood glucose

There is significant (P<0.0001) increase in the level of Blood glucose at group 2 when compared with Group 1. There is significant (P<0.001) decrease in the level of Blood glucose at group 3, 4 and 5 when Compared with group 2.

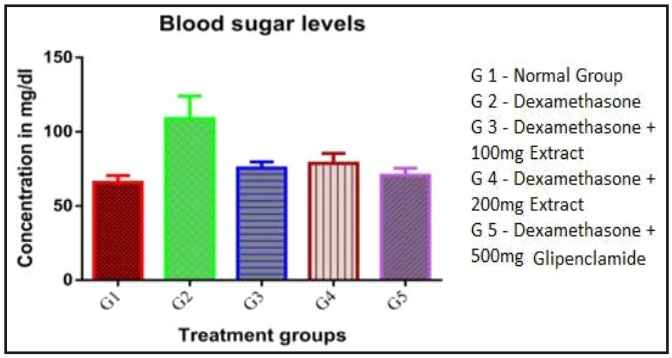

Figure-2: Effect of *Moringa Oleifera* on Blood Glucose Levels

Serum level of Total cholesterol

There is no significant increase in the level of Total cholesterol at group 2 when compared with group 1. There is significant increase (P<0.001) in the level of Total cholesterol at group 3 when compared to group 2. Group 4 showed significant increase (P<0.0001) in the level of total cholesterol compared to group2. There is significant(p<0.001) increase in the level of total cholesterol in group 5 compared to group.

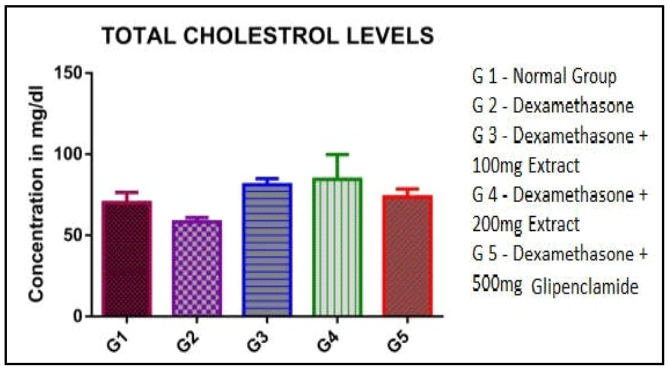

Figure-3: Effect of *Moringa Oleifera* on Total Cholesterol Levels

Effect of *Moringa Oleifera* on HDL

There is significance (p<0.01) increase in the level of ALP at group 2 when compared with group 1. There is significant (p<0.0001) decrease in the level of ALP at group 3 when compared to group 2. There is significance (p<0.01) increase in the level of ALP at group 4 when compared to group 2. There is no significance increase in the level of ALP at group 5 when compared to group 2.

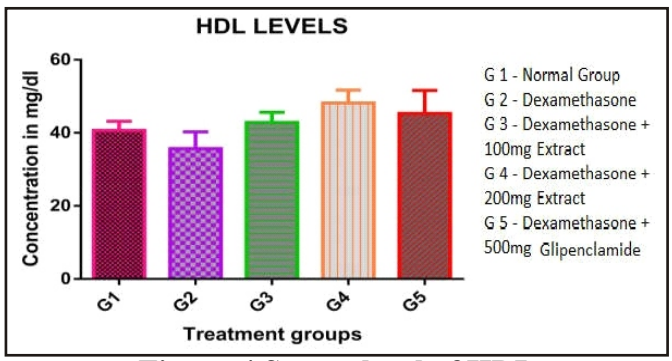

Figure-4 Serum level of HDL

Serum level of LDL

There is no significance increase in the level of LDL at group 2 when compared with group 1. There is significant increase (p<0.0001) in the level of LDL at group 3 when compared to group 2. There is significance (p<0.01) increase in the level of LDL at group 4, when compared to group 2. There is significance (p<0.0001) increase in the level of LDL at group 5 when compared to group 2.

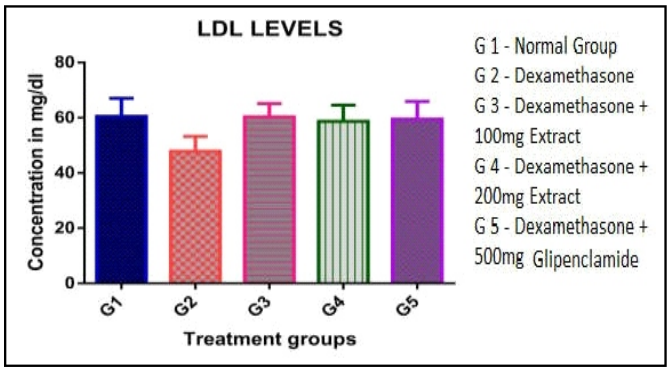

Figure-5 Serum level of LDL

Serum level of VLDL

There is no significance increase in the level of VLDL at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of VLDL at group 3, 4 and 5 when compared with group 2.

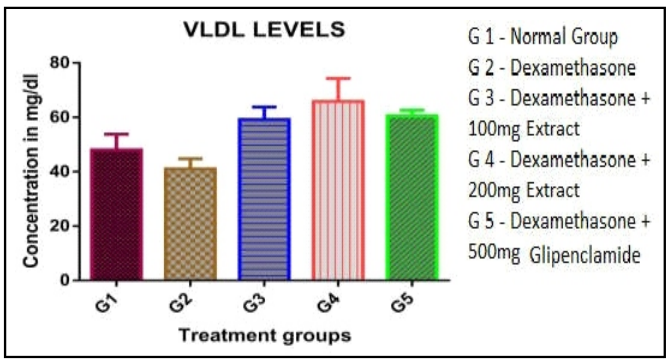

Figure-6 Effect of *Moringa oleifera* on VLDL level

Serum level of Triglycerides

There is no significance increase in the level of Triglycerides at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of cholesterol at group 3, 4 and 5 when compared with group 2.

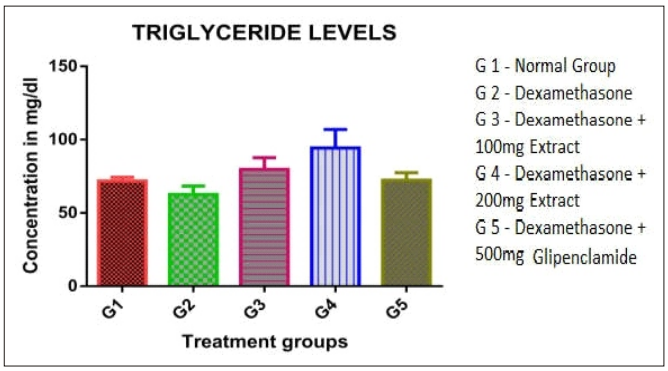

Figure-7 Serum level of Triglycerides

Effect of *Moringa oleifera* on tissue parameters

Pancreas are homogenized and TBARS, GSH, SOD, Catalase, levels were estimated.

The results were below.

Effect on TBARS

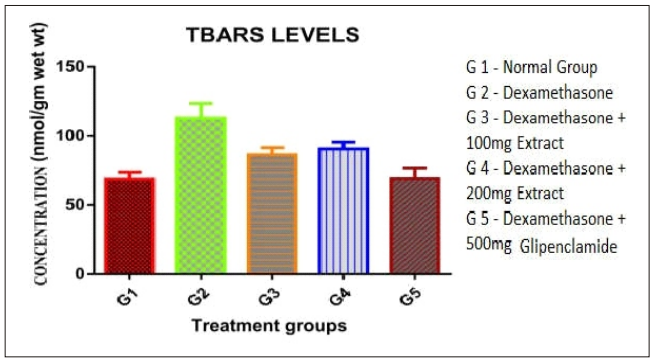

Figure-8 Effect of *Moringa oleifera* on TBARS levels

There is significance ($P<0.0001$) increase in the level of TBARS at group 2 when compared with group1. There is significance ($P<0.01$) decrease in the level of TBARS at group 3, 4, and5 when compared with group 2.

Effect on reduced glutathione (GSH)

There is significance decrease in the level of GSH at group 2 when compared to group There is significance ($P<0.0001$) increase in the level of GSH at group 3 when compared to group 2. There is significance ($P<0.0001$) increase level of GSH at group 4, 5 when compared with group 2.

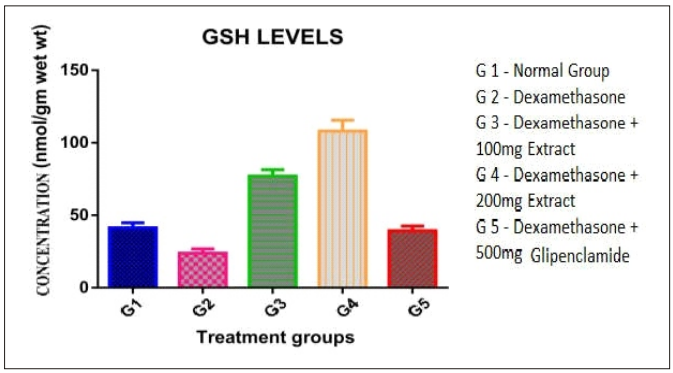

Figure-9 Effect of *Moringa oleifera* on GSH levels

Effect on superoxide dismutase (SOD)

There is significance decrease in the level of SOD at group 2 when compared with group 1. There is significance (P<0.0001) increase in the level of SOD at group 3,4 and 5 when compared with group 2.

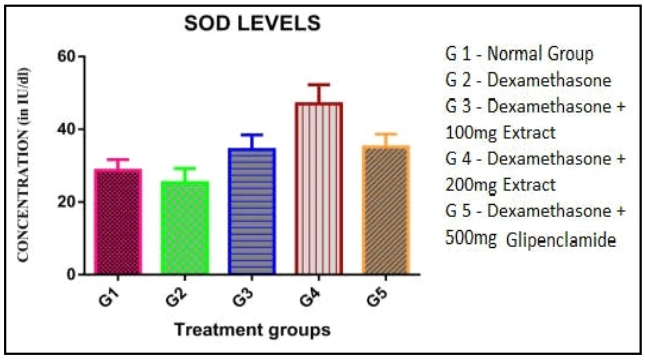

Figure-10 Effect of *Moringa oleifera* on SOD levels

Effect on Catalase (CAT)

There is significance decrease in the level of catalase at group 2 when compared to group 1. There is significance (p<0.0001) increase in the level of catalase at group 3 when compared with group 2. There is significance (P<0.0001) increase level of catalase at group 4, 5 when compared with group 2.

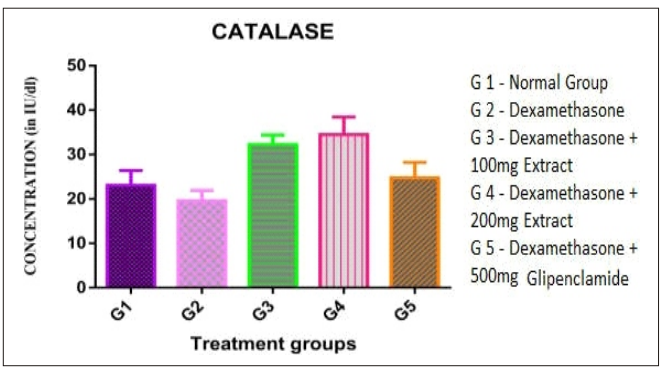

Figure-11 Effect of *Moringa oleifera* on Catalas

Conclusion

In current research, we investigated the effect of *Moringa Oleifera* on dexamethasone-induced insulin resistance models. Various biochemical estimates such as glucose, cholesterol. Triglycerides, LDL and HDL levels and antioxidant estimates such as TBARS SOD, CAT and GSH were estimated in two different doses of *Moringa Oleifera* (100 and 200 mg / kg p.o) and then compared with the standard and induced compound.

Moringa Oleifera at a dose of 100mg/kg and 200mg/kg prevented the development of hyperglycemia, hypercholesteremia and hypertriglyceridemia in dexamethasone induced insulin resistance models. Oral administration of *Moringa Oleifera* 100mg/kg and 200mg/kg reduces serum glucose, triglyceride, total cholesterols and LDL concentration and improve the concentration of HDL in dexamethasone administered rats. The lignin *Moringa Oleifera* showed significant anti-diabetic effect in rats after oral administration.

The present study demonstrated that *Moringa Oleifera* could be useful in Management of diabetes associated with abnormalities in lipid profiles. Further study needs to isolate, identify the active compounds and find out the possible mechanism of actions.

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About Flowers (Shown on the Cover Page)
Herbal Immunobooster

Syzygium aromaticum

Kingdom	:	Plantae
Division	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	Syzygium
Species	:	S.aromaticum

Medicinal Uses

- Cloves are the dried flower buds of the Syzygium Aromaticum tree. For years, cloves have widely been used not only as a spice but also as medicine for many ailments. It was traditionally used for curing tooth decay, digestive issues, bad breath, and even as an aphrodisiac.
- Cloves improve digestion by stimulating enzyme secretions and increase the digestive motility.
- The amazing ingredient, eugenol in clove is very effective against many harmful bacteria, fungus, and virus. The anti-viral and blood purification potential of clove decrease the toxicity in blood and increase the resistance against diseases by stimulating white blood cells.

Mentha arvensis

Kingdom	:	Plantae
Division	:	Spermatophytina
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	Mentha
Species	:	Menthaarvensis

Medicinal Uses

- The fresh leaves of ***Pudina*** have a unique aroma and taste which makes it a popular choice in different cuisines. The oil made of ***Pudina*** is very aromatic and is usually called as oil of spearmint which is widely used as a flavoring agent in toothpaste.
  Pudina is used in treating respiratory problems like asthma, cold, bronchitis, nasal congestion, sinus and congestion in the lungs.
- ***Pudina*** leaves also relieve sore throat, headache, chest congestion and runny nose.
  Pudina is used to reduce fever and neuralgia.
- It nurtures the skin, reduces pain and inflammation.

Tinospora cordifolia

Kingdom	:	Plantae
Division	:	**Magnoliophyta**
Class	:	Magnoliopsida
Order	:	Ranunculales
Family	:	Manispermaceae
Genus	:	Tinospora
Species	:	Menthaarvensis

Medicinal Uses

- This herb activated the immune system of our body and increase vitality in a person. Include Giloy juice or kadha in your diet twice a day can improve your immunity. It is full of antioxidants and helps to release toxins from the body. Giloyjuices also detoxifies your skin and improve your skin. Giloy is also used for liver diseases, urinary tract infections, and heart-related issues.
- Giloy is an antipyretic herb. It improves platelet count in dengue fever and reduces the chances of complications. Regular intake of Giloy helps to improve immunity during dengue and also for a speedy recovery. For better results boil Giloy juice with few Tulsi leaves and drink to increase platelet count.

Piper nigrum

Kingdom	:	Plantae
Division	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Piperales
Family	:	Piperaceae
Genus	:	Piper
Species	:	P.nigrum

Medicinal Uses

- Black pepper is great in aiding the proper assimilation of food. Moreover, its outer layer which contains potentphytonutrientsstimulates the breakdown of fat cells. This is the reason, why this spice ranks high among the best weight loss aids.
- Piperine present in black pepper stimulates the taste buds to signal the stomach to produce more hydrochloric acid. This acid is essential to digest proteins and other foods in the stomach, which if left undigested cause flatulence, indigestion, diarrhoea, constipation and acidity. The excess hydrochloric acid secreted helps in preventing these conditions.

Forth Comming Events

ICABB 2021: 15. International Conference on Applied Bioscience and Biotechnology
January 07-08, 2021 in Tokyo, Japan

International Conference on Drug Development, Metabolism and Phytochemistry ICDDMP on January 11-12, 2021 in Singapore, Singapore
Website URL: **https://waset.org/drug-development-metabolism-and-phytochemistry-conference-in-january-2021-in-singapore**

International Conference on Drug Development and Phytochemistry ICDDP on January 21-22, 2021 in London, United Kingdom
Website URL: https://waset.org/drug-development-and-phytochemistry-conference-in-january-2021-in-london

ICBMP 2021: 15. International Conference on Biochemistry and Molecular Pharmacology
February 04-05, 2021 in Bangkok, Thailand
DIGITAL

5th Annual Meet on Nano Science and Nanotechnology
February 22-23, 2021 Webinar

11th International Conference on **Herbal Medicine and Acupuncture**
March 22-23, 2021 Webinar

4th International Conference on Natural Products and Medicinal Plants Research
September 24-25, 2021 Montreal, Canada

7th International Conference on Natural, Traditional & Alternative Medicine
June 07-08, 2021 Vancouver, Canada

4th International Conference on Natural Products and Medicinal Plants Research
September 24-25, 2021 Montreal, Canada

14th World Congress on Food Chemistry and Food Microbiology
April 05-March 23, 2021 Webinar

7th Annual Congress on Plant Science and Molecular Biology
May 18-19, 2021 Webinar

5th International Congress on Traditional Medicine, Therapies and Modern Health Care
June 21-22, 2021 Tokyo, Japan

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Council Initiative for promotion of reverse pharmacology in Ayurvedic drug development

Uttarakhand State Council for Science & Technology (UCOST) was established in the last quarter of 2005 in Dehradun. Since its inception council has encouraged the research and development activities in the state and has funded projects in various disciplines of Science & Technology. Council provides financial assistance in R&D, International Travel supports, Entrepreneurship Development Program (EDP), Seminar/Symposium/Conference/Workshop grants etc. In innovation promotion programthe grassroots level for application/ invention catering local needs and all individuals with demonstrable talent are being promoted. The council aims to forge partnership between Central and State Governments, NGOs, R & D institutions, academia and industry, Council will act as hub, maximizing collaboration between various organizations and promote science in multidisciplinary mode. As an initiative, Coordination Cell of the Council are being set up at various institutions.The council has established a state-of-art Regional Science Centre in Uttarakhand sponsored by NCSM, Kolkata, catering to needs of the people of state especially school going children. The regional science Centre will also have an innovation lab sponsored by National Innovation Council.

As far as medical science is concerned, UCOST has initiated an ambitious “Drug Development” program to promote drug development in Ayurveda within the ambit of reverse pharmacology and the guidelines laid down by WHO for the development of natural products. We have recently reviewed promising therapeutic effects of Herbo-mineral Formulations for prophylaxis of Chronic Pancreatitis and migraine, Faltrikadi kwath for prophylaxis of Hepatitis B and started to facilitate advanced R&D following reverse pharmacology. We are intended to work on drug development for some tropical diseases in near future under Drug Development program provided that the aspiring Vaidya or Ayurvedic traditional healers have maintained meticulous record of their clinical work.

I extended my best wishes to Universities’ Journal of Phytochemistry and Ayurvedic Height for their endeavor in Herbal research.

Dr. Rajendra Dobhal
Director General

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