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# Universities' Journal of Phytochemistry and Ayurvedic Heights

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## Herbs for Treating Diabetes mellitus

*Syzygium jambolana (Jamun)*

*Aegle marmelos (Bel)*

*Balsamodendron mukul (Guggul)*

*Ocimum sanctum (Tulsi)*

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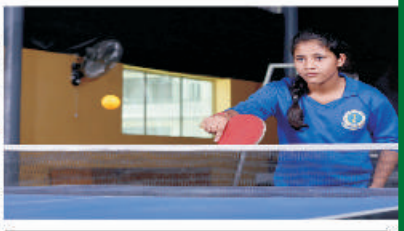

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**Dr. I.P. Pandey,** Co-ordinator
Universities' Journal of Phyto-Chemistry and Ayurvedic Heights,
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# Editorial

Dear Readers,

Greetings! India's immediate challenge is reviving its economy and job creation. Developing Herbal Pharma Industry is the best way by opening new units to create employment by our young scientists.

India's Pharmaceutical Market is expected to expand to US $55 Billion by 2020 and going to be the largest market globally. This is due to lower cost of Production as compared to US and almost half cost when compared with Europe. As we all know that the Indian Pharmaceutical Market is the third largest in terms of Volume and thirteen largest in terms of value. Pharma Industry in India is expected to grow 15% Per Annum by 2020 and it will outperform to Global Pharma Industry which is set to grow at an annual rate of 5% during the same period.

Government of India has given its nod for FDI up to 100% under the automatic route of manufacturing of Medical Devices subject to certain conditions. None of the Ayurvedic firm is listed amongst the major Investment holders.

Ayurveda in its strides for curing incurable diseases eg.- Diabetes and Cancer (Karkat) provides a bastion, preserving principles or activities by our seers like Charak, Susrat, Kanad and Kapil etc.

In the process one generation throws the gauntlet to another. This will continue till we succeed in unlocking secrets of nature in diverse fields through profound research demanding deep study and thought.

The Pharmaceutical Department has planned to launch a venture Capital Fund of Rs.1000/- Crores to support starts up in the R&D in Pharmaceutical and Biotech Industry.This shows a ray of hope in Indian endeavors which will help in furtherance of glitter and glory to our herbal wealth.

My sincere thanks to all those who have contributed for bringing out this Issue and my special thanks to Dr. Rajendra Dobhal (DG, UCOST) who always stood for the cause like my younger brother and Departmental Heads of Universities and other officials who deserve gratitude for the support rendered in our endeavor by sending students and staff. At the end, I express my gratitude to all Board members, guests who released this issue, Media & Staff of UCOST.

**Dr. S. Farooq**
*Chief Editor*

# Diabecon, an Ayurvedic Formulation Exhibits Antiglycation Effect *in Vitro*

**Faizan Abul Qais[1], Iqbal Ahmad[1*] and S. Farooq[2]**
[1]Department of Agricultural Microbiology,
Aligarh Muslim University, Aligarh, UP, 202002. India
[2]Himalaya Drug Company, Dehradun, Uttarakhand, 248001. India
*E-mail: ahmadiqbal8@yahoo.co.in

**Abstract-** Hyperglycaemia is the hallmark of diabetes that increases the pathophysiological complications in diabetes. Under hyperglycaemia, proteins react with sugars both enzymatically and non-enzymatically to form advanced glycation end products (AGEs) *via* a series of complex reactions. The AGEs formed under *in vivo* conditions generate free radicals and induces oxidative stress to various tissues and organs and thereby amplifies the diabetic complexities. Inhibition of glycation is one of strategy to avoid such complications. Various antidiabetic drugs, both modern and herbal medicines may exhibit varying level of antiglycation effect as one of the antidiabetic mechanism. However, many herbal formulations have not yet systematically explored for their mode of action on this aspect. In this study, we have investigated a polyherbal Ayurvedic formulation for Diabetes (Diabecon) for its potency in inhibiting protein glycation. BSA samples were incubated for 28 days with glucose in absence and presence of varying concentration (200-1000 µg/ml) of Diabecon under physiological conditions. The results showed that Diabecon significantly inhibited non-enzymatic glycation and protected BSA from unfolding. Inhibition of AGEs and fructosamine under *in vitro* conditions was recorded. These results highlight the role of Diabecon in inhibition of glycation and in turn preventing disease progression. The findings also validate the antidiabetic activity of Diabecon and proposed that its antiglycation mode of action may significantly contribute to its overall efficacy.

**Keywords**: Diabecon, BSA, Glycation, AGEs, Fructosamine, Diabetes

## Introduction

Diabetes mellitus is a metabolic disorder which is characterized by abnormal carbohydrate, fat and protein metabolism that ultimately affects insulin secretion as well as insulin action (Alam *et al*., 2016). Earlier, the disorder was regarded as disease of the rich and affluent while this has become a chronic disease irrespective of geographic location and socioeconomic status. It is one of the leading causes of death in developing and developed countries producing a substantial evidence of becoming an epidemic mainly in developing nations (Whiting *et al*., 2011). Hyperglycaemia is hallmark of diabetes whether type I or type II. Elevated blood sugar causes glycation of large number of proteins in which human serum albumin (serum protein) is mainly affected. Glycation is a reaction of reducing sugars like glucose and fructose with protein and nucleic acids that give rise to an unstable Amadori products or Schiff's base (Qais *et al*., 2016). Proteins are more prone to glycation than nucleic acids. These unstable glycation intermediates further follow complex cascade reactions that include condensation, rearrangements, oxidative modifications causing the abnormal cross-linking of proteins and ultimately forms advanced glycation end products (AGEs) (Brownlee, 2001). Under *in vivo* conditions, methylglyoxal, glyoxal and dicarbonyls are major classes of AGEs formed. The accumulation of AGEs in body tissue leads to increase in pathophysiological complications associated with diabetes. This stimulates the production of reactive oxygen species (ROS) causing massive tissue damage.

The herbal medication has recently attained a considerable popularity and acceptance

worldwide (Goldbeck-Wood *et al*., 1996). In the last few years, there was up to two-fold increase in use of herbal formulations as medicines that had led to the development of standard guidelines for detailed study of their pharmacotherapeutic and pharmacodynamic properties (Margolin *et al*., 1998). There has been remarkable advancement in understanding of diabetes and its causes, but the modern synthetic drugs have not proven to be satisfactory in management of this disease that often linked to cause undesirable effects (Agrawal and Kar, 2013). Herbal therapy is believed to impose no or very less side effects for both short term and long term administration.

There are a number of monoherbal and polyherbal formulations available to manage complications associated with diabetes in which polyherbal formulations has more evidences of better therapeutic efficacy (Sarasa *et al*., 2012). Diabecon is a polyherbal formulation of several herbs used for the management of diabetes and its associated complexities. The major key component of this drug includes various herbal ingredients as depicted in Table-1. Studies conducted on this formulation have proved to be a potent antioxidant and antidiabetic (Jyoti *et al*., 2013). It has been shown that Diabecon prevented diabetic complications and reduced oxidative stress in different tissues of adolescent rats (Agrawal and Kar, 2013). A study conducted on diabetic subjects showed that it only reduced the blood sugar level and dosage of conventional antidiabetic drugs but also proved efficient in management of microalbuminuria and blood lipids (Malhotra, 1999). Another phase III clinical trial study found that administration of this drug improved the hepatic glycogen and prevented the oxidative damage of beta cells by increasing superoxide dismutase activity of the islet cells (Kohli *et al*., 2004). The treatment of albino rats with Diabecon achieved a euglycemic state and slowed down the progression of diabetic nephropathy (Mitra *et al*., 2010). Several other clinical and non-clinical studies have found the therapeutic potential of Diabecon and found to be an effective formulation in the management of diabetes and related pathophysiological problems (Kundu and Chatterjee, 2010 and Moghaddam ae al., 2005). Various antidiabetic drugs both modern and herbal medicines may exhibit varying level of antiglycation effect as one of the antidiabetic mechanism. However, many herbal formulations including Diabecon have not yet systematically explored for their mode of action through this mechanism. In the present study, we have evaluated the antiglycation potential of Diabecon, an Ayurvedic formulation, using bovine serum albumin (BSA) as model protein.

## Material and Methods

Diabecon tablets (uncoated) were obtained as gift from Himalaya Drug Company, (Dehradun unit), India. The tablets were dissolved in distilled water and were diluted for further use. BSA and glucose was purchased from Hi-Media Laboratories, Mumbai, India. Nitro blue Tetrazolium Chloride (NBT) was obtained from SRL chemicals, India.

### In Vitro Glycation Assay

The antiglycation assay of Diabecon was performed by the method adopted by Qais *et al*. (2016) with few modifications (Qais *et al*., 2016). Bovine serum albumin (BSA) was incubated with D-glucose in absence and presence of Diabecon for 28 days at 37 °C in which the final concentration of BSA and glucose was 300 µM and 150 mM respectively. Aminoguanidine (10 mM) was used in place of DIABECON as positive control. Approximately, 0.02% (w/v) $NaN_3$ was added to each sample to avoid microbial contamination. All dilutions and preparations were made in 10 mM phosphate buffer saline (PBS) until and unless stated. On completion of incubation, all the samples were extensively dialyzed for 48 hrs against same buffer to remove unbound glucose and other compounds. All samples were stored at -20 °C for further analysis. Freshly prepared BSA of the same concentration was taken as native control.

### UV-Vis Spectroscopic Analysis

All UV-vis spectroscopic analysis was performed

on Cintra 10*e* (GBC Scientific Equipment Ltd., Australia). All samples were diluted to 10 µM in 10 mM PBS and absorbance spectra were recorded in the rage of 200 to 700 nm. PBS was used for baseline correction. The percent hyperchromicity was calculated using formula (Raghav *et al*., 2017),

$$\% \text{ hyperchromicity} = \frac{Abs_t - Abs_n}{Abs_g} \times 100$$

where, $Abs_t$, $Abs_n$ and $Abs_g$ are the absorbance of treated, native and glycated at 280 nm.

**Measurement of fluorescent of AGEs**

The relative amount of fluorescent AGEs all samples were assayed by spectrofluorometer (RF-5301, Shimazdu, Japan). Each sample was diluted to 10 µM in phosphate buffer prior to the fluorescence measurement. Samples were excited at 370 nm and emission spectra were recorded in the range of 380-600 nm. The percent inhibition in fluorescent AGEs were calculated using following formula

$$\% \text{ Inhibition} = \frac{FI_g - FI_t}{FI_g - FI_n} \times 100$$

where, $FI_g$, $FI_t$ and $FI_n$ are the fluorescence intensity of glycated, treated and native BSA samples.

**Estimation of fructosamine using Nitro blue tetrazolium dye**

The amount of fructosamine in samples was quantified using NBT assay (Johnson *et al*., 1983). Native, glycated and treated HSA (50 µl each) was mixed with 3 ml of 250 µM NBT reagent (in 0.1 M carbonate buffer of pH 10.5) and reaction mixture was incubated for 2 hours at 37 °C. The absorbance of the colour developed was recorded spectrophotometrically at 525 nm. The Amadori product was quantified taking 12640 $cm^{-1}$ $mol^{-1}$ as molar extinction coefficient for monoformazone (Alok *et al*., 2016).

## Results and Discussion

**UV-Vis Spectroscopy**

The preliminary examination for structural changes of various BSA samples were carried out by UV- visible spectroscopy. Native BSA and other samples showed absorbance maxima at 280 nm in which absorbance of BSA was least which is shown in **figure-1**. Increase in absorbance is

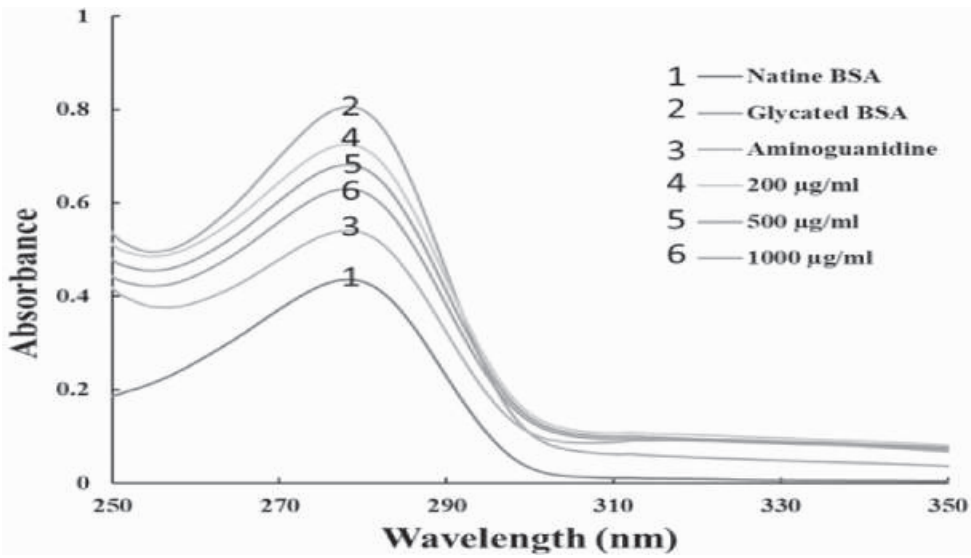


**Figure-1 UV-visible spectra of native, glycated and Diabecon treated BSA after 28 days. (aminoguanidine is positive control).**

**Table-1 Major Key plants found in diabecon tablets**

| S.No. | Name of plant | Common/Hindi name |
|---|---|---|
| 1.0. | *Eugenia jambolana* | Jamun |
| 2.0. | *Gymnema sylvestre* | Gurmar |
| 3.0. | *Pterocarpus marsupium* | Vijaisar |
| 4.0. | *Ocimum sanctum* | Tulsi |
| 5.0. | *Aloe vera* | Kumari |
| 6.0. | *Triphala* | Triphala |
| 7.0. | *Aegle marmelos* | Bel |
| 8.0. | *Terminalia arjuna* | Arjun |
| 9.0. | *Balsamodendron mukul* | Guggulu |

**Table-2 Demonstrating percent hyperchromicity, percent AGEs inhibition and fructosamine level (µmol/mg protein) in bovine serum albumin upon non-enzymatic glycation with at varying concentrations of Diabecon.**

| | % Hyperchromicity | % AGEs inhibition | Fructosamine level (µmol/mg protein) |
|---|---|---|---|
| **Native BSA** | - | - | 7.71 |
| **Glycated BSA** | 45.86 | - | 49.20 |
| **Aminoguanidine** | 12.96 | 81.66 | 14.79 |
| **Diabecon (200 µg/ml)** | 35.75 | 19.70 | 39.85 |
| **Diabecon (500 µg/ml)** | 30.45 | 43.94 | 32.39 |
| **Diabecon (1000 µg/ml)** | 24.07 | 68.32 | 23.37 |

termed as hyperchromicity. Glycated BSA sample showed 45.86% hyperchromicity as compared to native BSA. It is evident from the data presented in **Table-2** that treatment at 200, 500 and 1000 µg/ml of Diabecon decreased the hyperchromicity by 35.75, 30.45 and 24.07 %. Aminoguanidine, a positive control, showed minimum hyperchromicity of 12.96 %. The relatively more absorbance of different samples might be due to formation of AGEs and their aggregates, as a result of cross-linking (Guerin-Dubourg *et al*., 2012). It has been observed that glycation of protein leads to unfolding that in turn exposes cyclic ring structure electrons causing the

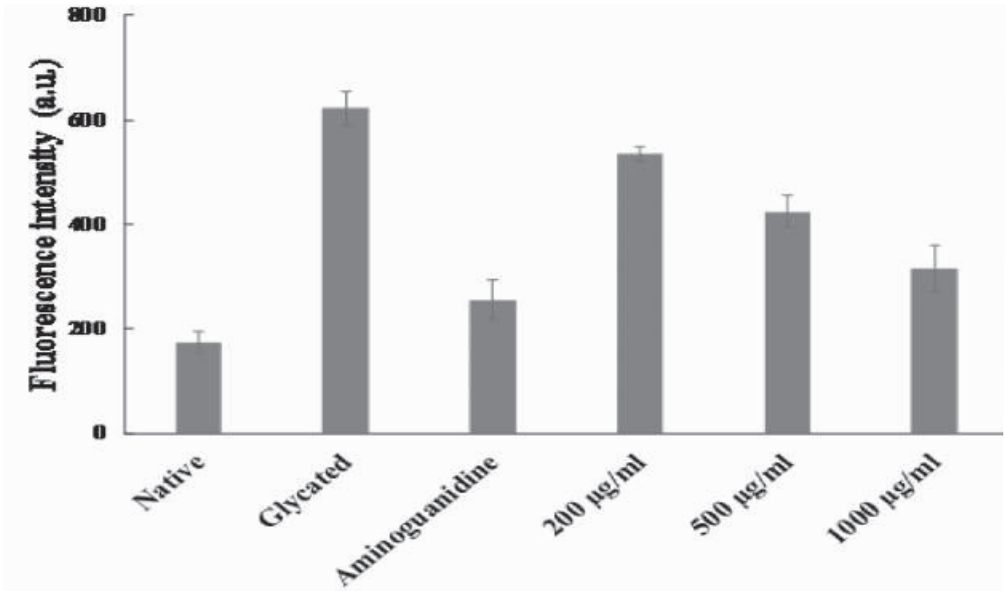


**Figure-2 Fluorescence emission of native BSA, glycated BSA, BSA treated with different concentrations of Diabecon.**

hyperchromic effect to protein. Another reason for hyperchromicity is due to unfolding of globular structure of protein as well as reshuffling of bonds (Moosavi-Movahedi et al., 2003). The result indicated that Diabecon effectively protected BSA from unfolding.

**Estimation of Fluorescent AGEs**

AGEs are a major group of heterogenous compounds which is a key factor in pathogenicity of several diabetes related problems. BSA exhibits weak fluorescence when excited at 370 nm, while AGEs showed significantly high fluorescence when excited at same wavelength. Therefore, AGEs specific fluorescence was employed for their detection. The fluorescence intensity of native, glycated and treated BSA samples are shown in **Figure-2**. It is clear from data that glycation resulted in approximately four-fold increase in fluorescence intensity. Treatment of varying concentration of Diabecon significantly decreased the AGEs specific fluorescence. The protective effect of Diabecon is presented in terms of percent inhibition in **Figure-3** and data is presented in **Table-2**. It can be seen that there was 70 % inhibition in the formation of fluorescent AGEs. The fluorescence in BSA is mainly due to presence of two tryptophan amino acids. When BSA gets glycated, the microenvironment of the amino acid residues (mainly fluorophore) are altered resulting in increased fluorescence. It has also been bound that glycation causes the opening of the hydrophobic domains of serum albumin that contributed to the florescence (Qais *et al*., 2016). Our findings elucidate the dose dependent inhibitory effect of this Diabetic formulation and protected the formation of AGEs.

**Fructosamine assay by NBT**

BSA samples incubated with D-glucose in absence and presence of varying concentration of Diabecon were quantitatively evaluated for fructosamine (a major Amadori product) content using NBT assay. Amadori products are first group of stable end products after formation of Schiff's base of glycation reaction (Qais *et al*., 2016). The level of fructosamine in different samples is shown in **Figure-4**. The amount of fructosamine in native BSA was found to be 7.71 µmol/mg protein that increased to more than thirteen folds (49.20 µmol/mg protein) in glycated

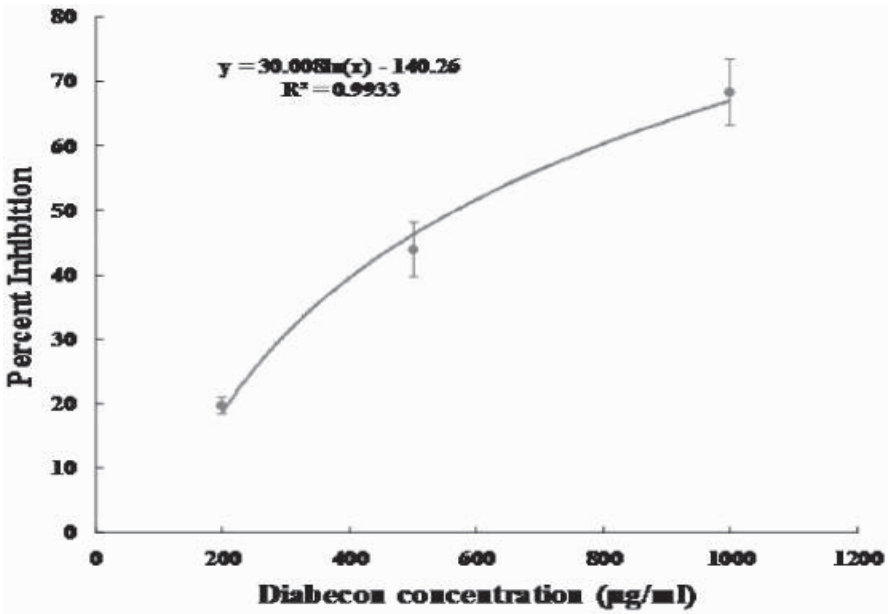


**Figure-3 Inhibitory effect of Diabecon on formation AGEs.**

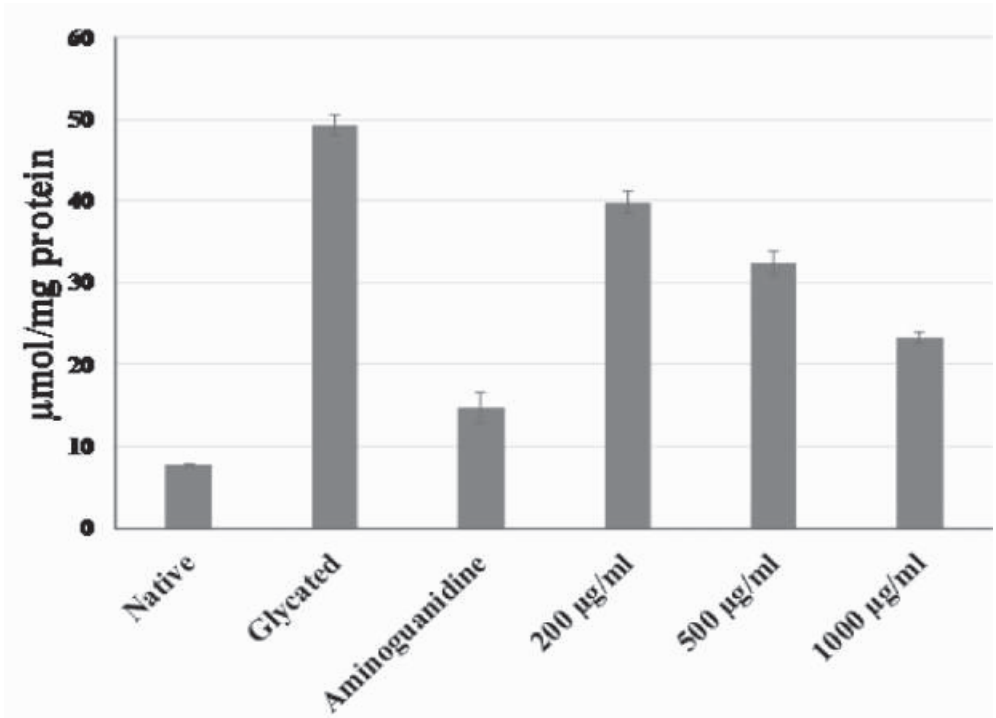


**Figure- 4 Level of fructosamine in native BSA, glycated BSA and Diabecon treated BSA estimated by NBT assay.**

sample. On treatment of 200, 500 and 1000 µg/ml of Diabecon, the fructosamine level decreased to 39.85, 32.39 and 23.37 µmol/mg protein. The result demonstrated that there was approximately 50% inhibition in fructosamine formation at 1000 µg/ml treatment. The level of fructosamine in human samples has been directly correlated to duration of hyperglycaemia within the patients (Reusch *et al*., 1993). Therefore, it may be concluded that Diabecon effectively checks the level of fructosamine and that may be a mechanism of Diabecon to prevent diabetes associated complications.

Ingredients of Diabecon increases insulin secretion through insulin secretagogue and insulinomimetic actions of *Pterocarpus marsupium*, *Aloe vera* and *Ocimum sanctum*. *Gymnema sylvestre* stimulates endogenous insulin secretion by altering the cell membrane permeability.

Effective hyperglycemic control is crucially important to prevent micro and macrovascular complications of diabetes mellitus. *Gymnema sylvestre* ,Triphala, *Eugenia jamboolana* and tulsi decrease hepatic glucose production and prevent hyperglycemia. *Pterocarpus marsupium* has hypoglycemic action and reduces glucose absorption from the GI tract.

## Conclusion

In conclusion, the results of the present study, decipher the antiglycation potential of Diabecon in BSA-glucose model. The treatment of varying level of this herbal formulation prevented serum albumin from unfolding and exposure of hydrophobic domain that occurs in case of prolonged hyperglycaemia. It also, successfully inhibited the formation of AGEs which causes many diabetic complication and induces oxidative stress. The fructosamine content was also found to be lowered in presence of this antidiabetic drug. It can be said that Diabecon effectively inhibits the glycation reaction and might be quite helpful in preventing complications associated with the increased blood sugars. Further studies in animal model and human subjects are needed to validate and find the exact underlying mechanism.

## Conflict of Interest

The authors declare there are no conflicts of interest.

## Acknowledgements

We are grateful to Director, Himalaya Drug Company, Dehradun, India for providing uncoated Diabecon tablets and Dr. Zafar Mehmood for help in preparing this manuscript.

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# Antifungal Potential of *Berberis aristata* Against Phytopathogens

**Rashmi[1*], Sapna Bhardwaj[2], Y.P.Singh[3] and Archana Bhagwari[4]**
[1,2]Chemistry Division, Forest Research Institute, Dehradun,
Uttarakhand, P.O. New Forest-248006, India
[3,4]Pathology Division, Forest Research Institute, Dehradun,
Uttarakhand, P.O. New Forest-248006, India,
*E-mail: rashmi@icfre.org and sehrawat82@gmail.com

**Abstract-** Plant metabolites and plant based fungicides appear to be one of the better alternatives in plant disease management, as they are known to have minimal harmful impact on the environment and danger to consumers in contrast to the synthetic fungicides. Therefore, the present study was carried out to screen roots of *B. aristata* for its antifungal potency against phytopathogenic fungi. The preliminary **qualitative phytochemical analysis was also carried out and results** showed the presence of alkaloids, phenolics and tannins, flavanoids, sterols, protein and amino acids etc. The *in-vitro* antifungal activity of root extracts was carried out by Poisoned food technique against phytopathaogenic fungi viz. **Rhizoctonia solani, Sclerotium rolfsii and Fusarium solani.** Results revealed that methanol extract at concentration 10% showed 100% inhibition against *S. rolfisii*, while against *R. solani* and *F. solani* showed 71.4% and 50% inhibition respectively at 15% concentration. Therefore, methanol extract was found to be most effective against these phytopathaogens where $IC_{50}$ value could be achieved.

**Keywords**: *Berberis aristata*, Qualitative analysis, Phytopathogens, Antifungal activity.

## Introduction

***Berberis aristata***, commonly known as **Indian barberry** or **tree turmeric**, is a shrub belonging to the family Berberidaceae; genus *Berberis*. The genus comprises approximately 450-500 species of deciduous evergreen shrubs and is found in the temperate and sub-tropical regions of Asia, Europe, and America. *B. aristata* is native to the Himalayas in India and Nepal. It is also naturally found in the wet zone of Sri Lanka. The plant is widely distributed from Himalayas to Srilanka, Bhutan, and hilly areas of Nepal in Himalaya region (Sharma et al. 2011). It grows at the height of 2000-3000 m especially in Kumaon and Chammba region of Himachal Pradesh. It is also found in Nilgris hills in South India (Sabharn, 1964; Shah and Joshi, 1971; Chauhan,). It is one of the economically important and of high medicinal value species of temperate areas. It is an erect, glabrous spinescent shrub, 3–6 m in height with obovate, subacute and entired leaves (Anonymous, 1981). Its stem, roots and fruits are being used in many Ayurvedic preparations since a long time.

The plant is used traditionally in inflammation, wound healing, skin disease, diarrhea, jaundice and infection of eyes. The plant show hepatoprotective, antitumour, sedative and wound healing properties. Alkaloids, terpenoids, flavanoids, sterols, anthocyanins, vitamins and carotenoids have been characterized from different parts of the plant. A very valuable Ayurvedic preparation'Rashut' is prepared by this plant which is used in curing human ailment like ophthalmic, ulcer, as laxative and tonic and blood purifier (Kurian, 2007; Parmar and Kaushal, 1982). The dried berries are edible. The fresh berries are laxative and antiscorbutic and useful in piles, sores and eye diseases, particularly conjunctivitis. A decoction is used as a mouthwash for treatment of swollen gums and toothache. The plant fruit is edible and it is rich in vitamin C. Pharmacological studies on the plant

reveal the proven activity of its as hypoglycemic, antibacterial, antifungal, antipyretic, anti-inflammatory, hepatoprotective, antioxidant, and anticancer (Sharma et al. 2011). Phytochemical studies shows that plant *B. aristata* contains mainly yellow colored alkaloids berberine, oxyberberine, berbamine, aromoline, karachine, palmatine, isotetrandrine and jatrorrhizine, oxycanthine and taxilamine and tannins, sugar, starch (Atta-ur-Rahman and Ansari, 1983; Gilani and Janbaz, 1995). Berberine is one of the important marker alkaloidal active principles of this plant (Rashmi et al. 2008).

Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides (Kin et al. 2005). The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987). Although there is a growing interest in the use of medicinal plants to control the plant diseases, only about 2,400 plant species among more than 250,000 higher plants have been screened for the phytoactivity (Oluwalana and Adekunle, 1998; Oluwalana et al. 1999; Khafagi et al. 2000). The plant based pesticides are cheap, locally available, non-toxic, and easily biodegradable. Plant metabolites and plant based pesticides appear to be one of the better alternatives in plant disease management, as they are known to have minimal harmful impact on the environment and danger to consumers in contrast to the synthetic pesticides (Verma and Dubey, 1999). Therefore, the present study was carried out to screen the different extracts of *B. aristata* for their antifungal potency in laboratory conditions against important phytopathogenic fungi viz. ***Rhizoctonia solani, Sclerotium rolfsii*** **and** ***Fusarium solani.***

## Material and Methods

### Collection of Plant Material

The roots of *Berberis aristata* were collected from Sarhan (Shimla) and authenticated by Head, Botany Division, Forest Research Institute, Dehradun, India. A voucher specimen has also been submitted to Botany Division, Forest Research Institute, Dehradun, India.

### Preparation of Root Extracts

The freshly collected roots (1kg) of *B. aristata* were shade dried, cut into small pieces and coarsely powdered. The powdered plant material (570 g) was defatted with petroleum ether (60-80$^0$C). The defatted material was extracted with the solvents of elutropic series viz. chloroform and methanol by using soxhlet apparatus and aqueous methanol extracts were prepared by refluxing sequentially. Each time before extracting with the next solvent of high polarity, the powdered roots were dried. Each extract was concentrated by distilling off the solvent, which was recovered subsequently. Extracts were evaporated to dryness and their yield percentage was calculated on moisture free basis (Table-1).

### Preliminary Qualitative Phytochemical Analysis

The different extracts of *B. aristata* root was subjected to qualitative phytochemical analysis for the presence or absence of plant constituents such as alkaloid, glycosides, carbohydrates, phenolics and tannins, flavonoids, saponins and triterpinoids. The analysis was carried out by following the standard methods (Trease and Evans, 1989; Sofowora, 1993; Harbone 1998).

### Screening of Antifungal Activity

The different extracts prepared from *B. aristata* root were tested for their antifungal activity against three common forest fungi, namely *Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium solani.* Poisoned Food Technique was used to test the antifungal activity (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and

Thapilyal, 2002). The sterilized potato dextrose agar (PDA) medium was poured into the sterile petri dishes and allowed to solidify. A culture of test fungi of disc (0.7 cm dia.) was grown on PDA for about seven days. Requisite amount of extracts was dissolved in respective solvents to prepare 5%, 10% and 15% concentration of extracts. Suitable check was maintained where the culture discs were grown under same conditions on PDA without extract. Inoculated petri plates were incubated at 25 $^{0}\pm$ 1 $^{0}$ C. The experiment was carried out in triplicates and the data was recorded every day. The percentage inhibition of fungal growth was estimated (Vincent, 1927).

## Results and Discussion

570 g of dried and powdered root of *B. aristata* was extracted using solvent of elutropic series viz. petroleum ether, chloroform, methanol and aqueous methanol. Highest percentage yield (3.21%) was obtained for aqueous methanol extract (Table-1). Preliminary phytochemical analysis was carried out of different extracts (Table-2). Results revealed that the alkaloids, phenolics and tannins, flavanoids, sterols, protein and amino acids are found to be present in methanol extract. While aqueous methanol extract also showed the presence of carbohydrates, glycosides and saponins in addition to other constituents. The petroleum ether and chloroform extracts were rich in triterpenoid, fixed oil and fats.

The *in-vitro* antifungal activity of chloroform, methanol and aqueous methanol extract was carried out against three phytopathaogenic fungi at different concentrations. Results showed that with the increase in concentration, the growth inhibition percentage of all the test fungi also increases. Among all the three extracts, methanol extract showed highest activity and results are presented in Table-3, 4 and 5. Methanol extract at concentration 10% showed 100% inhibition against *S.rolfisii*, while against *R. solani* and *F. solani* showed 71.4% and 50% inhibition respectively at 15% concentration (Figure). Chloroform extract shows moderate activity against *R. solani* (57.1%) and *S. rolfisii* (50%). While aqueous methanol extract was found to be least active against all the test fungi.

## Conclusion

The study showed that the *B. aristata* root was rich in secondary metabolites such as alkaloid, phenolics and tannins, flavanoids, sterols, protein, aminoacids, saponins and terpenoids. Methanol extract of root was found to be most active against the test fungi, where $IC_{50}$ could be achieved. It has potential to inhibit the growth of *S. rolfisii* completely. Therefore, *B. aristata* root could be developed as herbal fungicide against the plant pathogens. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity.

**Table-1 Yield (%) of different extracts of *Berberis aristata* root.**

| S.N. | Extract | Weight (g) | Yield (%) |
|---|---|---|---|
| 1. | Hexane | 1.45 | 0.30 |
| 2. | Chloroform | 2.85 | 0.58 |
| 3. | Methanol | 13.01 | 2.63 |
| 4. | Aqueous methanol | 15.80 | 3.21 |

**Table-2 Preliminary qualitative analysis of Different extracts of *Berberis aristata* root.**

| S.No | Phytoconstituents | Pet. Ether | Chloroform | Methanol | Aqueous methanol |
|---|---|---|---|---|---|
| 1. | Alkaloids | - | + | + | + |
| 2. | Carbohydrates | - | - | - | + |
| 3. | Glycosides | - | - | - | + |
| 4. | Phenolics and tannins | - | + | + | + |
| 5. | Flavonoids | - | + | + | + |
| 6. | Saponins | - | - | - | + |
| 7. | Triterpenoids | + | + | - | - |
| 8. | Fixed oils and fats | + | + | - | - |
| 9. | Sterols | - | _ | + | + |
| 10. | Proteins and amino acids | - | + | + | + |

+ = Present, – = Absent

**Table-3 Growth Inhibition (%) of test fungi against chloroform extract of *B. aristata* root.**

| Fungus | Concentration / Growth Inhibition (%) | | | | |
|---|---|---|---|---|---|
| | Control | Check | 5% | 10% | 15% |
| *R. solani* | 0 | 0 | 38.6 | 47.1 | 57.1 |
| *S.rolfisii* | 0 | 0 | 21.4 | 40.1 | 50.0 |
| *F. solani* | 0 | 0 | 23.6 | 28.6 | 40.7 |

**Table-4 Growth Inhibition (%) of test fungi against methanol extract of *B. aristata* root.**

| Fungus | Concentration / Growth Inhibition (%) | | | | |
|---|---|---|---|---|---|
| | Control | Check | 5% | 10% | 15% |
| *R. solani* | 0 | 0 | 57.1 | 60.0 | 71.4 |
| *S.rolfisii* | 0 | 0 | 48.6 | 100 | 100 |
| *F. solani* | 0 | 0 | 21.4 | 30.0 | 50 |

**Table-5 Growth Inhibition (%) of test fungi against aqueous methanol extract of *B. aristata* root.**

| Fungus | Concentration /Growth Inhibition (%) | | | | |
|---|---|---|---|---|---|
| | Control | Check | 5% | 10% | 15% |
| *R. solani* | 0 | 0 | 27.1 | 37.1 | 57.1 |
| *S.rolfisii* | 0 | 0 | 4.3 | 17.1 | 35.7 |
| *F. solani* | 0 | 0 | 14.3 | 25.7 | 30.0 |

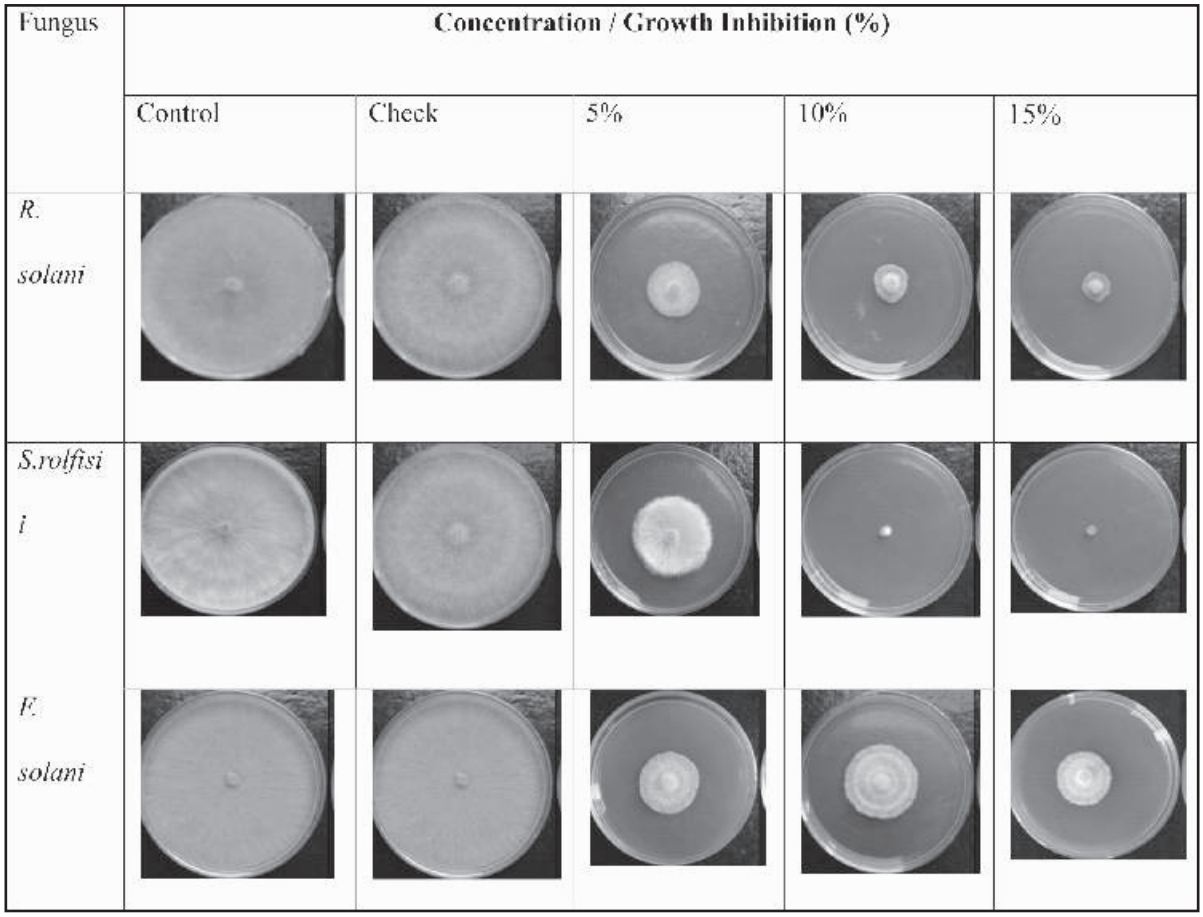


**Figure Growth Inhibition (%) of test fungi against methanol extract of *B. aristata* root.**

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# Pharmacognostic Evaluation and Physico-Chemical Analysis of *Peltophorum pterocarpum*

**Kundan Singh Bora* and Pooja Pandey**
Department of Pharmacognosy, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand–240001, India
*E-mail: kundan1381@gmail.com

**Abstract-** *Peltophorum pterocarpum* (family: Leguminosae) has been traditionally used in the treatment of many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm, dysentery, embrocation for pains and sores. The present investigation establishes the pharmacognostic and physico-chemical parameters for *P. pterocarpum.* Transverse section of *P. pterocarpum* stem showed that the outermost layer consisted of a single layer of epidermis cells. Inner epidermis included three or four layer of cortex. The xylem segments possessed several circular thick walled vessels and the pith having several hexagonal cells. The powdered leaf of *P. pterocarpum* showed the presence of paracytic stomata, xylem vessel, parenchyma cell and spiral vessel. Physico-chemical parameters like ash values and extractive values of various extracts were also determined. In phytochemical screening, various extracts of *P. pterocarpum* shows the presence of different phyto-constituents such as carbohydrates, flavonoids, phenols and tannins. Fluorescence analysis of powdered drug and extract showed the different fluorescence in UV. In conclusion, the outcomes of pharmacognostic evaluation and physico-chemical analysis of leaf and stem of *P. pterocarpum* could be helpful for identification and authentication of the plant. It may also serve as a reference material in the preparation of herbal monograph.

**Keywords**: Evaluation, Microscopy, *Peltophorum pterocarpum,* Pharmacognostic, Transverse section.

## Introduction

Standardization of natural products is a complex task due to their heterogeneous composition which is in the form of whole plant, plant part or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Reddy *et al.,* 1999; Venkatesh *et al.,* 2004). Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity of such material. It should be carried out before any tests are undertaken.

*Peltophorum pterocarpum* (DC) Backer ex K. Hayne is a herbaceous plant in the family leguminosae, It is widely grown in tropical region of South Eastern Asia, Sri Lanka, Thailand, Indonesia, Malaysia, Papua New Guinea, Philippines and the islands of the coast of Northern Territory, Australia (Huxley, 1992; Rasingam *et al.,* 2012). The plant is also found in different regions of India including Birbhum District, West Bengal. The wood of the plant has wide variety of uses, including cabinet-making and the foliage is used as a fodder crop. It is deciduous tree growing to 15-25 m tall, with a trunk diameter upto 1m (McCann, 1966). Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles,

constipation, ringworm and its flower extract is known to be a good sleep inducer and used in insomnia treatment (Burkill, 1995; Siri *et al.,* 2008). Its bark is used as medicine for dysentery, as eye lotion, embrocation for pains and sores. The traditional healers use the leaves in the form of decoction for treating skin disorders. Stem infusion of *P. pterocarpum* is used in dysentery, for gargles, tooth powder and muscular pain. Flowers are used as an astringent to cure or relieve intestinal disorders, after-pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores (Bheemachari *et al.,* 2005; Duraipandayan *et al.,* 2006; Satish *et al.,* 2007; Valdlapudi and Naidu, 2010).

The current investigations were aimed to evaluate the pharmacognostical features and physico-chemical analysis of *Peltophorum pterocarpum* for identification and authentication of the plant.

## Material and Methods

### Collection and Identification of Plant Material

Plant material was collected from the campus of Sardar Bhagwan Singh PG Institute of Biomedical Sciences and Drug, Balawala, Dehradun India, in the month of February, 2017. The plant was authenticated at Forest Research Institute, Dehradun, India.

### Macroscopic Study

Fresh parts of *P. pterocarpum* were used for cutting transactional sections with the help of using different suitable reagents. Powder microscopy was done by the Dutch process. Two gram of powder of selected part was taken to which was added 10% nitric acid solution (50 ml). This solution was warmed for 2 mins. It was filtered and residues so obtained was washed with hot water and then filtered. Then again residues were taken to which 10% sodium hydroxide solution (50 ml) was added and warmed for 2 mins. The solution was again filtered. The residue was washed with hot water and again filtered. Finally, the residue for powder microscopy using a student microscope (Olympus) (Khandelwal, 2006)was taken.

### Fluorescence Analysis

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. In this dried powdered drug was treated with various reagents, which showed characteristic fluorescence at 254nm and 365nm (Khandelwal, 2008).

**Physico-Chemical Parameters (**WHO, 2007, Khandelwal, 2008**)**

### Determination of Ash Values

### Total Ash

2 g of the powdered drug was accurately weighed in a tarred crucible (platinum or silica).The material was spread in an even layer in the crucible and placed in muffle furnace to iginite at a temperature not exceeding 450ºC until it was white, indicating that it was free from carbon. The crucible was cooled in desiccator and weighed. The total ash was calculated as the percentage of ash with reference to the air-dried plant material.

### Acid-insoluble Ash

The total ash obtained from the procedure mentioned above was boiled for 5 minutes with 25ml of hydrochloric acid. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the crucible, dried on a hot plate and ignited in muffle furnace for 15 minutes at a temperature not exceeding 450ºC. The content of acid-insoluble ash was calculated as a percentage of ash with reference to the air-dried plant material.

### Water-soluble Ash

The total ash obtained from the procedure mentioned above was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash-less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450ºC. The total weight of insoluble matter was subtracted from the weight of the ash. This difference in weights represented

the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

**Determination of Extractive Values**

This method determines the amount of active constituents extracted with different solvents from a given amount of plant drug. The extraction of any drug with a particular solvent yields a solution containing different phyto-constituents. The use of a single solvent can be the means of providing information on the quality of a particular drug sample.

**Petroleum Ether Soluble Extractive**

5 g of coarsely powdered (leaves) accurately weighed and air-dried material was placed in a glass-stopper conical flask.100ml of petroleum ether was added and weighed to obtain the total weight including the flask. It was shaken well and gently boiled for 6 hours at temperature 60-70°C, then it was transferred to a tarred flat-bottomed china dish and evaporated to dryness on a water-bath at 105°C, cooled in a dessicator for 30 minutes, then weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

**Ethyl acetate soluble extractive**

5 g of coarsely powdered (leaves) separately accurately weighed and air dried material was placed in a glass-stopper conical flask. 100 ml of ethyl acetate was added and weighed to obtain the total weight including the flask. It was then shaken well and gently boiled for 6 hours at temperature 60-70°C and then transferred to a tarred flat-bottomed china dish and evaporated to dryness on a water-bath at 105°C, cooled in a dessicator for 30 minutes, then weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

**Chloroform Soluble Extractive**

5 g of coarsely powdered (bark) accurately weighed air-dried material was placed in a glass-stopper conical flask. 100 ml of chloroform was added to it and weighed to obtain the total weight including the flask. It was then shaken well and gently boiled for 6 hours at temperature 60-70°C, then transferred to a tarred flat-bottomed china dish and evaporated to dryness on a water-bath at 105°C, cooled in a dessicator for 30 minutes, then weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

**Ethanol Soluble Extractive**

5 g of coarsely powdered (bark) accurately weighed air-dried material was placed in a glass-stopper conical flask. 100ml of ethanol was added to it and weighed to obtain the total weight including the flask. It was shaken w**ell and** gently boiled for 6 hours at temperature 60-70°C, then transferred to a tarred flat-bottomed china dish and evaporated to dryness on a water-bath at 105°C, cooled in a dessicator for 30 minutes, then weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

**Water Soluble Extractive**

5.0g of coarsely powder (**leave)** of ***P. pterocarpum***, accurately weighed air-dried material was placed in a glass-stopper conical flask.100ml of water was added to it and weighed to obtain the total weight including the flask. It was shaken **well and** gently boiled for 6 hours at temperature 60-70°C, then transferred to a tarred flat-bottomed china dish and evaporated to dryness on a water-bath at 105°C, cooled in a dessicator for 30 minutes, then weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material

**Phytochemical Screening** (Khandelwal, 2008)

All the extracts viz., petroleum ether, chloroform, ethyl acetate, methanol and water were subjected to preliminary phytochemical screening for the detection of various phyto-constituents such as alkaloids, glycosides,

tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrate and triterpenoids.

## Results

### Macroscopy of Leaf

Odour: None; Taste: Sweet; Shape: Oblong; Margin: Entire; Apex: Obtuse

Base: Asymmetric

### Macroscopy of Bark

Colour:Grayish brown; Odour: None; Surface: Smooth; Fracture: Hard

### Microscopy

Transverse sections of *P. pterocarpum* stem are shown in Figure-1A, 1B and 1C.

Transverse sections of *P. pterocarpum* stem are shown in Figure-1A, 1B and 1C.

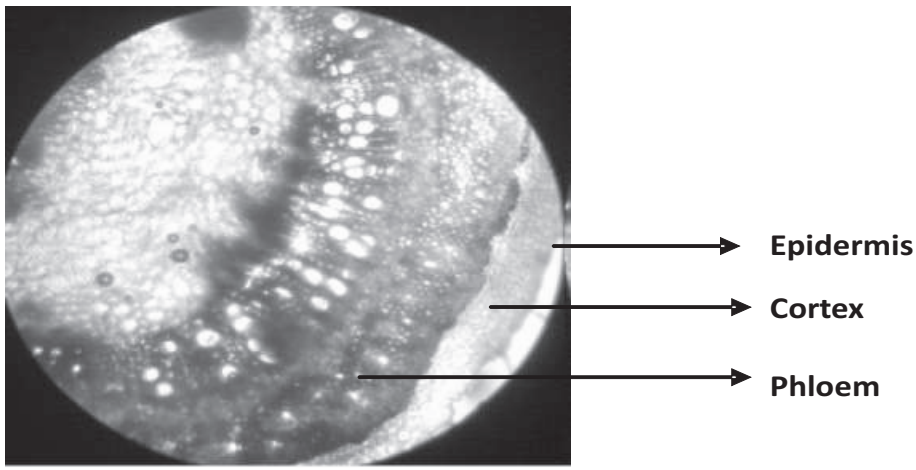


**Figure-1A**

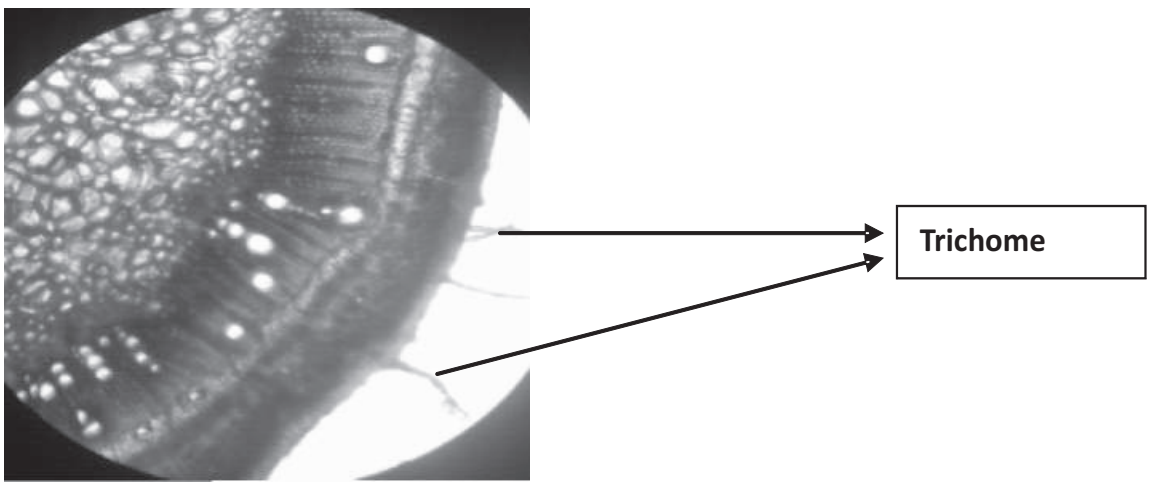


**Figure-1B**

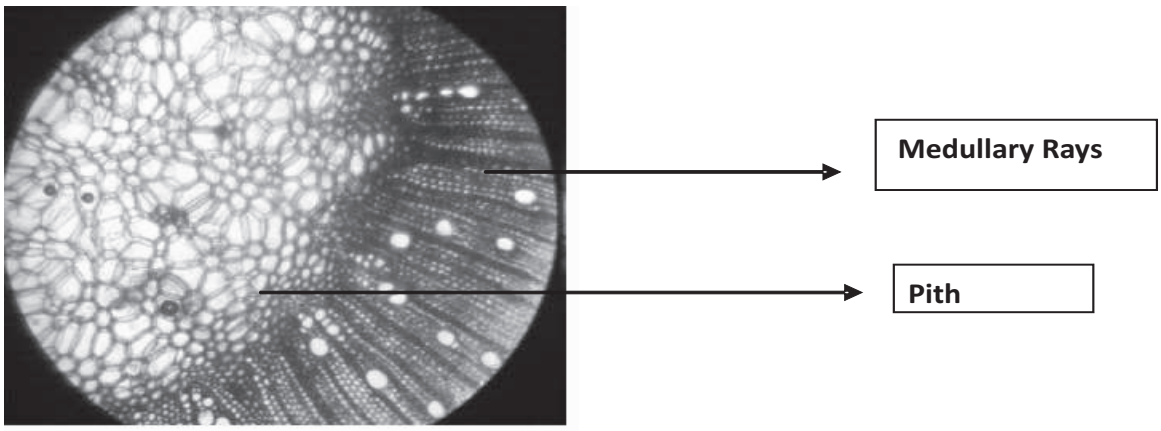


**Figure-1C**

**Power microscopy of *P. pterocarpum* leave shown in Figure-2A, 2B, 2C, 2D, 2E and 2F.**

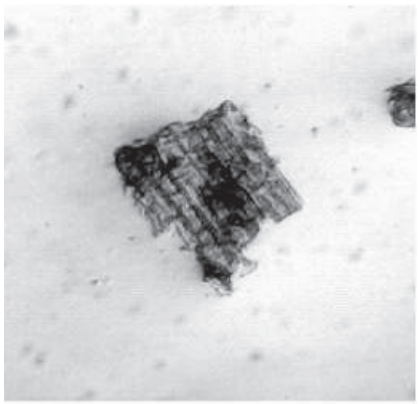

**Figure-2A Vessel**

**Figure-2B Covering Trichome**

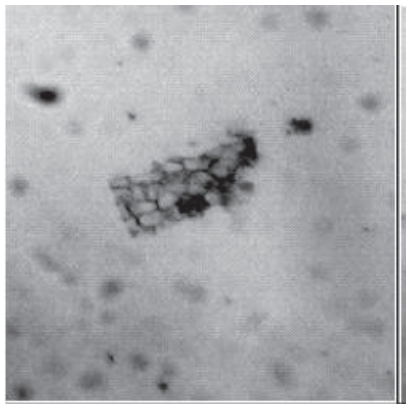

**Figure-2C Parenchyma cell**

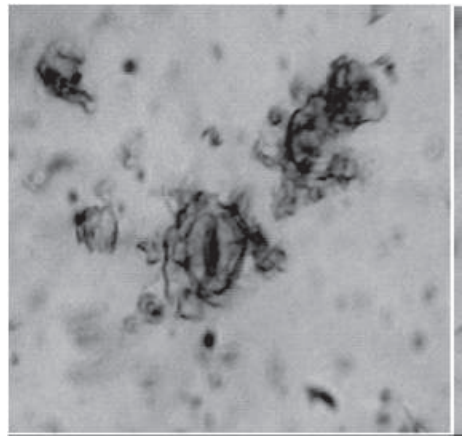

**Figure-2D Paracytic Stomata**

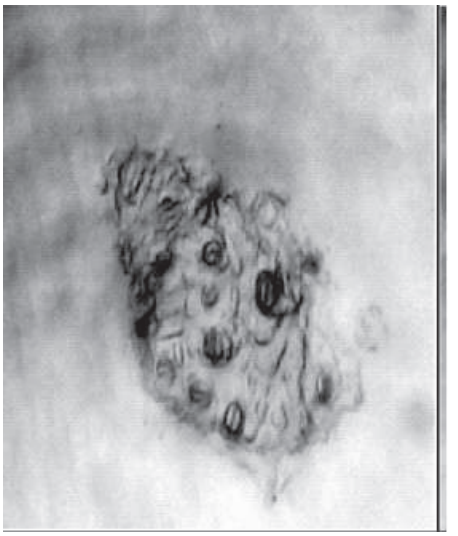

**Figure-2E Epidermis cell with Stomata**

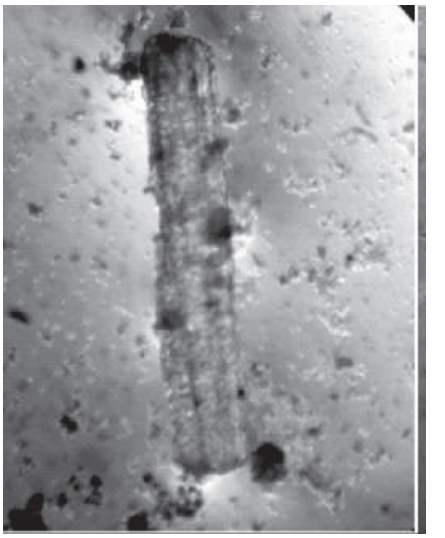

**Figure-2F Xylem Vessel**

**Table-1 Extractive yield, consistency and fluorescence analysis of *P. pterocarpum* (leaves)**

| Extracts | Yield (%w/w) | Consistency | Fluorescence analysis Under UV lamp | |
|---|---|---|---|---|
| | | | Long UV | Short UV |
| Petroleum ether extract | 14.4% | Solid mass | Greenish brown | Brown |
| Chloroform extract | 4.4% | Solid mass | Dark violet | Brownish green |
| Ethyl acetate extract | 5.8% | Semi solid mass | Blackish brown | Greenish brown |
| Methanolic extract | 20.4% | Solid mass | Brick red | Yellowish green |
| Aqueous extract | 22.6% | Semi solid mass | Blackish | Violet brown |

**Physico-Chemical Analysis**

**(a) Table-2 Ash value of *P. pterocarpum* (leaves)**

| S. No. | Type of ash | Ash value (%w/w) |
|---|---|---|
| 1. | Total ash | 8.41% |
| 2. | Acid insoluble ash | 1.4% |
| 3. | Water soluble ash | 5.96% |

**(b)Table-3 Extractive value *P. pterocarpum* (leaves)**

| S. NO. | Extract | Extractive value (%w/w) |
|---|---|---|
| 1 | Petroleum ether | 2.5 |
| 2 | Chloroform | 5 |
| 3 | Ethyl acetate | 8 |
| 4 | Methanol | 10.7 |
| 5 | Water | 12 |

**(c)Table-4 Fluorescence analysis of *P. pterocarpum* (leaves)**

| S. No. | Reagents | Long UV (365 nm) | Short UV (254 nm) |
|---|---|---|---|
| 1 | Conc. $H_2SO_4$ | Bluish black | Yellowish green |
| 2 | Conc. HCl | Blue | Violet |
| 3 | Methanol | Brick red | Brick red |
| 4 | n-Hexane | Brick red | Blue |
| 5 | n-Butanol | Dark red | Dark red |
| 6 | Chloroform | Dark red | Brick red |
| 7 | Water | Light green | Light green |
| 8 | Acetic anhydride | Dark green | Dark red |
| 9 | Ammonia | Dark blue | Light green |
| 10 | Petroleum ether | Dark blue | Dark blue |

**Table-5 Phytochemical screening of the various extract of *Peltophorum pterocarpum* (leaves)**

| S. No. | Phytoconstituents groups | Results | | | | |
|---|---|---|---|---|---|---|
| | | Pet. Ether Extract | Chloroform Extract | Ethyl Acetate Extract | Methanol Extract | Aqueous Extract |
| 1. | Carbohydrates | - | - | - | + | + |
| 2. | Flavonoids | - | - | - | + | - |
| 3. | Alkaloids | - | - | - | - | - |
| 4. | Tannin and phenolics | - | - | - | + | + |
| 5. | Proteins and amino acids | - | - | - | - | - |
| 6. | Steroids | - | - | - | - | - |

## Discussion

The present study was emphasized on pharmacognostic evaluation and physico-chemical analysis of leaf and stem of *Peltophorum pterocarpum*. Macroscopic and microscopical studies of any herbal drug are the primary steps to establish its botanical quality control before going to other studies. As per WHO norms, botanical standards are to be proposed as a protocol for the diagnosis of herbal drug. Transverse section of *P. pterocarpum* stem showes that the outermost layer consists of a single layer of epidermis cells. Inner epidermis included three or four layer of cortex. The xylem segments possess several circular thick walled vessels and the pith having several hexagonal cells. The powdered leaf of *P. pterocarpum* shows the presence of paracytic stomata, xylem vessel, parenchyma cell and spiral vessel.

The physico-chemical standards, such as, ash values, extractive values and fluorescence analysis are useful to identify the drug even in the crushed or powdered condition. These also serve as a standard data for quality control of the preparations containing this plant in future. In this study ash values, extractive values and fluorescence analysis of various extracts were determined.

Phytochemical screening is also useful to isolate pharmacologically active principles present in the drug. The information obtained from ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species and varieties. In the present study various extracts of *P. pterocarpum* shows the presence of different phyto-constituent such as carbohydrates, flavonoids, phenols and tannins.

In conclusion, the outcomes of pharmacognostic evaluation and physico-chemical analysis of leaf and stem of *P. pterocarpum* could be helpful for the identification and authentication of the plant. It may also serve as a reference material in the preparation of herbal monograph.

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# *In vitro* Inhibitory Activity of Koflet Ingredients Against Pathogenic Bacteria

**S.Farooq, Arunesh Kumar Dixit and Zafar Mehmood***
*E-mail: zafarmehmood31@gmail.com

**Abstract-** Koflet syrup and Lozenges are phytopharmaceutical formulation recommended for cough of varied etiology. Cough is an annoying and exhausting symptom in a wide variety of respiratory disorders. Many of the presently available antitussive drugs have either a central depressing action or drying action on the respiratory secretions making expectoration of sputum more exhausting for the patient. Koflet has been found to be free from such effects. It was helpful in providing significant relief from the distressing symptoms of cough and associated problems. It also facilitated easy expectoration of sputum while soothing the respiratory passages.

In the present study, Koflet ingredients have been tested and found to have antibacterial action. Sore throat can be treated initially with local antiseptic and anti-inflammatory medications. Herbal lozenges and syrup of Koflet are supposed to have these actions. Koflet is multi ingredients phytopharmaceutical formulation. Studies have shown that the antibacterial activity of different solvents extracts of *Syzygium aromaticum Terminalia chebula, Cinnamomum zeylanicum, Glycyrrhiza glabra,* and *Myrsitica fragrans, Adhtoda vasica, Tinospora cordifolia, Solanum xanthcarpum,Ocimum sanctum, Embelia ribes,Vitis vinifera,Balsamodendron mukul and Acacia catechu* were found to be active against test pathogens such as *Staphylococcus aureus,E.coli,Pseudomonas aeruginosa and Salmonella enteritis* with a diameter of zone of inhibition ranged from 10 mm to 22mm.

**Keywords:** Koflet ingredients, Antibacterial activity, Phytopharmaceutical formulation

## Introduction

Cough, dry or wet, is a frequent symptom of cardiorespiratory disorders and is usually annoying and exhausting for most of the patients. Most of the cough suppressants depress the respiratory centres in the brain stem while others, due to their antihistamine content, dry the respiratory secretions making their removal by expectoration more difficult and exhausting for the patient. An ideal antitussive agent should have a peripheral soothing action on the respiratory passages, should not depress the respiratory centres and should help liquefy and expectorate the secretions blocking the bronchial passages. Few cough remedies with these characteristics are available at present. However, Koflet (Himalaya) is claimed to have such desirable features.

Koflet is a combination of various parts of indigenous plants; the principal ingredients and their main pharmacological actions reported in the literature are reproduced briefly below :

| Drug ingredient | Actions Claimed |
|---|---|
| *Solanum xanthocarpum(Kantakari)* | Relieves fever, cough and facilitates expectoration. |
| *Adhatoda vasica(Vasaka)* | Liquefies sputum, soothes the nerves, relieves irritating cough. |
| *Ocimum sanctum (Tulsi)* | Demulcent, antipyretic and anticatarrhal. |
| *Embelia ribes (Vidang)* | Useful in sore throat and cough. |
| *Vitis vinifera (Draksha)* | Demulcent, expectorant, useful in colds and chronic bronchitis. |
| *Glycyrrihiza glabra(Mulethi)* | Demulcent in inflammatory affections. Soothes bronchial irritation. |
| *Terminalia chebula* | Broad spectrum antimicrobial activity |
| *Balsamodendron mukul* | Antiseptic, anti-infective. |
| *Cinnamomum zeylanicum* | Antibacterial, antifungal activity and antioxidant activity |
| *Myristica fragrans* | Antibacterial and anti-inflammatory activity |
| *Acacia catechu* | Useful in respiratory tract infections |
| *Syzgium aromaticum* | Soothes the throat and and provides antitussive activity |
| *Tinospora cordifolia* | Improves the cell mediated and humoral immune response |

The present study was undertaken with the aim to find out the antimicrobial efficacy of Koflet ingredients against pathogens responsible for various respiratory disorders.

## Material and Methods

Koflet ingredients (plants material) were obtained from Himalaya Drug Company, Dehradun, India as well as from the surroundings of Dehradun city located in Uttarakhand, India. The plants were properly identified and authenticated.

### Extraction

The plants material were collected, washed, air dried in shade and grinded by mixer grinder. After grinding, 300 gms of plant material were extracted in 1.2 liters of different solvents (methanol, hexane, and water) separately three times at 40°C to 45°C for 6 hours. The organic solvent was filtered by whatman filter paper till clear solution was obtained. Solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 40°C and the semi solid crude extract was placed in a vacuum oven at 40°C for dryness. The crude extract was stored in air tight container at dark place[1].

### Screening for Antibacterial activity

The antibacterial activity was carried out by employing 24h cultures of *Staphylococcus aureus, Escherichia coli ,Pseudomonas aeruginosa* and *Salmonella enteritis*. Activity of aqueous methanolic and Hexane extracts of *different plants* was tested separately using Agar well diffusion method[2, 3,4,5,6]. The medium was sterilized by autoclaving at $121^0$C (15 lbs/$in^2$ ). About 30 ml of the Agar medium with the respective strains of bacteria was transferred aseptically in to each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in 6mm diameter well. Antibacterial assay plates were incubated at 37 ± 2 ◦C for 24h, The Ciprofloxacin solution was used as a positive control for antibacterial activity, and diameter of the zone of inhibition was measured.

## Results and Discussion

Table-1 showed the antibacterial activity of the crude aqueous, Hexane, and methanolic extracts of *different plants* on *Staphylococcus aureus, E. coli ,Pseudomonas aeruginosa and Salmonella enteritis*. The methanolic extract of *Cinnamomum zeylanicum(plate-1) and Terminalia chebula(plate-2)* showed the highest antibacterial activity with the diameter of zone of inhibition 22mm against *Staphylococcus aureus followed by Ocimum sanctum,Myristica fragrans(plate-4) Syzygium aromaticum(plate-3), Solanum xanthocarpum and Acacia catechu.* Methanolic extracts showed the highest zone in the range of 11-22 mm followed by Hexane extract in the range of 10-20mm followed by aqueous extract ranged between 10-18mm as depicted in table-1.

The results obtained in this study revealed antimicrobial efficacy of methanolic extracts of all major plant ingredients of Koflet Lozenges and syrup. The active components of these plants may be due to their high non polar compounds. This is similar to the findings of Ijeh et al[7]. but in contrast to the findings of Obi and Onuoha[8] who documented ethanol as the best solvent for the extraction of plant active substances of medical importance. Methanol extracts were the most potent of all the extracts suggesting that the active component must be a highly non polar compound.

The antimicrobial activities of these extracts (methanol and Hexane) appeared to be broad

spectrum since both the Gram-positive and Gram negative bacteria were sensitive to their inhibitory effects and validated the use of Koflet syrup and lozenges to control cough associated with acute and chronic upper respiratory tract infections,tonsillitis and pharyngitis.

Koflet can be considered a useful and safe adjunct for substantial relief of cough in various respiratory disorders.

Sore throat can be treated initially with local

antiseptic and anti-inflammatory medications. Herbal lozenges and Syrup of Koflet are supposed to have these actions. These multi ingredient herbal formulations with herbs such as *Syzygium aromaticum Terminalia chebula, Cinnamomum zeylanicum, Glycyrrhiza glabra,* and *Myrsitica fragrans, Adhtoda vasica,Tinospora cordifolia,Solanum xanthcarpum Ocimum sanctum,Embelia ribes,Vitis vinifera, Balsamodendron mukul and Acacia catechu.*

*Acacia catechu* is known for its antimicrobial properties. Studies have shown that the antibacterial activity of *Syzygium aromaticum* was found to be active against various oral pathogens such as *Streptococcus mutans*, *Actinomyces viscosus*, *P. gingivalis* and *P. intermedia*. The flavonoids present in this plant demonstrated potent growth inhibitory activity in those pathogens, which causes periodontitis[9]. In a study when the extract of *Terminalia chebula* was used as a mouth rinse, it significantly reduced the total bacterial count and the total streptococcal counts in the saliva[10]. *Elettaria cardamomum*, which is present in Koflet lozenges is known for its anti-inflammatory activity which is equivalent in action to indomethacin[11]. *Cinnamomum zeylanicum* has anti-inflammatory properties[12]. Sore throat is also caused by viruses. *Glycyrrhiza glabra* present in the formulation acts as an anti-viral agent [13]. *Vitis vinifera* has antioxidant activity and is proven to have free radical scavenging properties[14].

**Table-1 Antibacterial activity of Koflet ingredients**

| Drug ingredient | *Diameter of Zone of Inhibition(mm)* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Aqueous Extract** | | | | **Methanol Extract** | | | | **Hexane Extract** | | | |
| | *Sa* | *Ec* | *Pa* | *Se* | *Sa* | *Ec* | *Pa* | *Se* | *Sa* | *Ec* | *Pa* | *Se* |
| *Solanum xanthocarpum(Kantakari)* | 12 | 10 | NAD | NAD | 18 | 16 | 15 | 16 | 15 | 12 | 13 | 14 |
| *Adhatoda vasica(Vasaka)* | 10 | NAD | NAD | NAD | 17 | 12 | 13 | 15 | 14 | 14 | 12 | 15 |
| *Ocimum sanctum (Tulsi)* | 12 | NAD | NAD | NAD | 20 | 16 | 15 | 16 | 18 | 13 | 15 | 13 |
| *Embelia ribes (Vidang)* | NAD | NAD | NAD | NAD | 16 | 15 | 15 | 12 | 14 | NAD | NAD | NAD |
| *Vitis vinifera (Draksha)* | NAD | NAD | NAD | NAD | 12 | 10 | 11 | 10 | NAD | NAD | NAD | NAD |
| *Glycyrrihiza glabra(Mulethi)* | 11 | NAD | NAD | NAD | 18 | 15 | 15 | 16 | 12 | 15 | 12 | 15 |
| *Terminalia chebula* | 14 | 12 | 12 | 10 | 22 | 18 | 18 | 20 | 16 | 16 | 15 | 18 |
| *Balsamodendron mukul* | NAD | NAD | NAD | NAD | 14 | 12 | 14 | 14 | 15 | 12 | 13 | 12 |
| *Cinnamomum zeylanicum* | 14 | 14 | 12 | 11 | 22 | 16 | 18 | 18 | 20 | 18 | 21 | 20 |
| *Myristica fragrans* | 15 | 15 | 14 | 15 | 19 | 15 | 16 | 16 | 19 | 17 | 15 | 18 |
| *Acacia catechu* | 16 | 15 | 16 | 18 | 18 | 16 | 12 | 14 | 14 | 12 | 10 | 10 |
| *Syzgium aromaticum* | 15 | 14 | 12 | 15 | 19 | 14 | 17 | 18 | 20 | 18 | 15 | 18 |
| *Tinospora cordifolia* | 12 | 10 | 13 | 12 | 16 | 15 | 16 | 15 | 18 | 16 | 14 | 12 |

Sa; *Staphylococcus aureus*

Ec*; Escherichia coli*

Pa;*Pseudomonas aeruginosa*

Sa;*Salmonella enteritis*

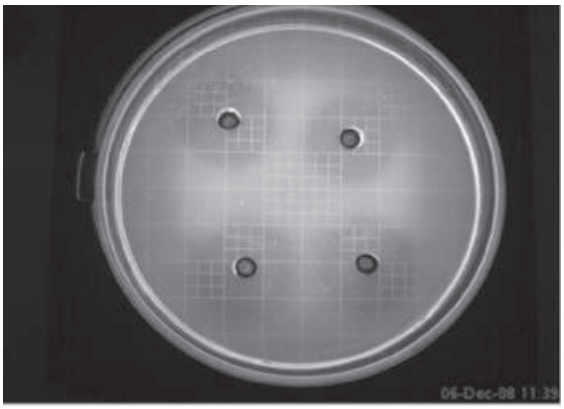

**Plate-1** Methanolic extract of *Cinnamomum zeylanicum* showed strong zone of inhibitions against Staph.aureus

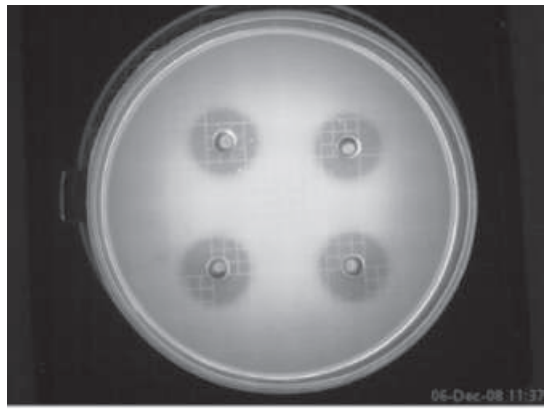

**Plate-2** Antibacterial activity of Methanolic extract of *Terminalia chebula* showed strong zone of inhibitions against Staph.aureus

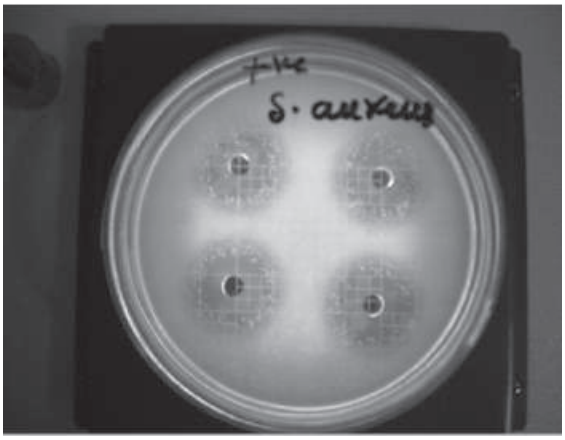

**Plate-3** Inhibitory activity Hexane extract of *Syzygium aromaticum* against *Staph.aureus*

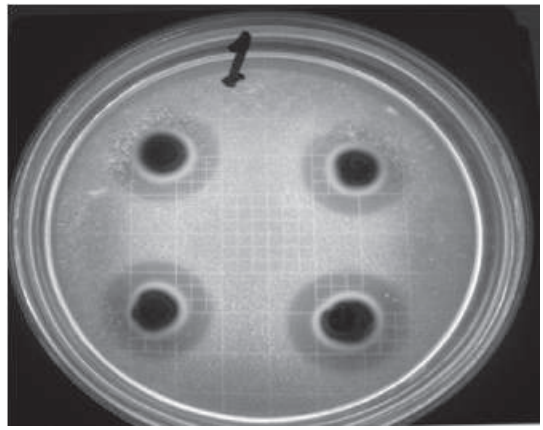

**Plate-4** Antibacterial activity of Methanolic extract of *Myristica fragrans* showed zone of inhibition against Staph.aureus

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# Evaluation of Efficacy and Safety of UNIM-215 in Experimental Animals

**Mohd Nadeem*, Mohd Urooj, Sadia Ayub, Habibur Rahman and Shariq Ali Khan**
Pharmacology Research Unit, Regional Research institute of Unani Medicine,
Aligarh-202002 U.P., India
*E-mail: nadeemmohd81@yahoo.com

**Abstract-** The present study was carried out to investigate efficacy and safety of UNIM-215 in albino mice and albino rats respectively. Efficacy of aqueous extract of UNIM-215 was determined in alloxan induced diabetes in albino rats with glibenclamide as a positive control. Four groups of rats were induced diabetes by administering alloxan at dose of 120mg/kgintraperitoneally. Out of the four groups one group was kept as control, the second group was given glibenclamide at dose of5mg/kg orally for nine days, while the third and fourth groups were given aqueous extract of UNIM-215 at dose of 0.5 gm/kg and 1.5gm/kg orally for nine consecutive days. The blood glucose levelwas estimated on 10th day revealed that the aqueous extract of UNIM-215 at both the dose levels significantly reduced the alloxan induced elevated blood glucose level.Safety study includes acute toxicity study and subacute toxicity study. Acute toxicity study was determined by administering aqueous extract of UNIM-215 orally to three groups of mice of six each at doses of 1gm/kg,3gm/kg and 5gm/kg body weight. The animals were observed for gross behaviour and mortality for 24 hours after drug administration. The formulation was well tolerated by the animals and noabnormality was observed in the general behaviour (salivation,lacrymation, lethargy,sleep and coma) of the animals and no mortality was recorded. Similarly, sub-acute toxicity was determined in albino rats by oral administration of aqueous extract of UNIM-215 to three groups of six animal each at the dose ranges from 0.5 gm/kg and 1.5gm/kg for 28 days. The results of hematology and biochemistry done on 29th day were found to be normal and no changes were observed in organ to body weight ratio of liver, heart, kidney and spleen.

**Keywords**: UNIM-215, Hypoglycemic activity, Acute toxicity, Subacute toxicity

## Introduction

Determination of efficacy and safety of herbal remedies is necessary because many people using these agents as self medication. There is limited data available about the safety of the commonly used herbal remedies and therefore effort to elucidate health benefits and risk of herbal medicine should be intensified (Anonymous, 2011).Toxicity testing in animal is typically the initial steps to determine the effect of test substances and potential hazards which occur due to short term exposure. A toxicity study provides information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity(OECD, 2000).

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency,chracterised by hyperglycemia,altered metabolism of carbohydrate,protein and lipid,and increased risk of vascular complications (Barar,2004).Diabetes mellitus is a global problem for which satisfactory treatment either in modern system of medicine or in traditional system of medicine is not available.Besides the use of synthetic medicines for the treatment of diabetes is associated with undesirable side effects (Berger,1985). Therefore, it has become imperative to search for hypoglycemic agents of plant origin which are safe and associated with less side effects.

UNIM-215 is a coded compound multi-ingredient herbal unani formulation prepared by CCRUM is claimed to be effective in hyperglycemia clinically. Since the hypoglycemic activity and toxicity of this drug has not been evaluated experimentally,hencethe hypoglycemic and toxicity studies of UNIM-215 were undertaken in albino mice and in albino rats respectively.

**Formulation**

UNIM-215 is a poly herbal formulation containing the constituents mentioned below

| S.No. | Common Name | Botanical Name |
|---|---|---|
| 1 | Maghz-e-Karela | *Momordicacharantia(Linn)* |
| 2 | MaghzKhastajamoon | *Syzygium cumin(Linn)* |
| 3 | Salajeet | Asphaltum |
| 4 | Kushta-e-Faulad | Iron |

## Material and Methods

**Setting**

This study was carried out in Pharmacology Research Unit (PRU) of Regional research institute of Unani medicine (RRIUM), Aligarh under Ministry of Ayush, Govt. of India.

**Ethical Consideration**

The study was conducted in accordance with the protocol approved by Institutional ethics committee, RRIUM, Aligarh, India.

**Procurement of Drug**

The UNIM-215 formulation was procured in the form of Tablet from Central research institute of Unani Medicine (CRIUM), A.G. Colony Road near ESI Hospital, Eragadda Road, Hyderabad, India

**Animals**

The study was carried out in Swiss albino mice (20-25 g) and rats (100-150g) of either sex, fordetermination of hypoglycaemic activity, acute and sub-acute toxicity respectively. The animals were procured from Mr. Rahat Hussain Enterprises Biological Suppliers, Babri Mandi,Aligarh. They were acclimatized to the conditions for one week before experimental study. The animals were maintained in a standard environmental condition at a room temperature of (25±2 °C) with 12 hrs light/dark cycles, humidity (50-55%) and had free access to food pellets. The study was conducted after approval of protocol from Institutional ethics committee of RRIUM, Aligarh.

**Preparation of Drug Extract**

The tablets of the drugs were crushed into fine powder and a weighed quantity was steeped in acidulated distilled water. The water soaked mass of the drug was warmed over water bath and kept for 24 hours, at room temperature. During this period, it was occasionally stirred. After 24 hours, it was filtered through a filter paper and filtrate was dried over water bath. The aqueous extracts of the drug thus obtained was used in different doses selected according to OECD guidelines for safety evaluations.

**Hypoglycemic activity**

Hypoglycemic activity was carried out on Swiss albino rats weighing around 100-150g. Albino rats of either sex were randomly selected and divided into four groups of six each.Diabetes was induced by injecting alloxan monohydrate dissolved in normal saline solution at a dose of 120mg/kg intraperitoneally to overnight fasted rats. The rats were then kept for next 24 hours on 10% glucose solution to prevent hypoglycemia. After 72 hours of alloxan injection, fasting blood glucose level was measured which increased significantly from the normal level.Out of the four groups, group I was kept as diabetic control which received distilled water orally, the group II rats was given glibenclamide at a dose of 5mg/kg orally, while group III and group IV rats received aqueous extract of UNIM-215 at dose levels of 0.5g/kg and 1.5g/kg orally for a period of nine days. The animals in all the four groups fasted overnight prior to blood collection. Blood samples were collected on 1st, 5th and 10th day for blood glucose estimation. Blood glucose was estimated according to orthotoluidine method. The results obtained revealed that glibenclamidesignificantly reduced the elevated blood glucose level after 4 and 9 days of treatment, while the drug at both the doses 0.5g/kg and 1.5g/kg brought down significantly the elevated blood glucose level after 9 days of treatment only (Table-1)

### Acute Toxicity Study

The current study was carried out in accordance to Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals. Swiss Albino mice of either sex weighing 20-25 g were randomly selected and divided into three groups of six mice each. Mice were kept fasted overnight (12hrs) with free access to water prior to administration of dosesat 1gm/kg, 3g/kg and 5gm/kg body weight orally.The animals were kept in polypropylene cages after drug administration and were observed for Gross behaviour (salivation, lacrymation, lethargy, sleep and coma) and mortality at 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 24 hours and thereafter, once every day up to 14 days after drug administration.

### Sub-Acute Toxicity Study

Swiss Albino rats of either sex weighing between 100-150 g were randomly selected and divided into three groups of six animals each. Rats were kept fasted overnight (12hrs) with free access to water prior to administration of dose ranging 0.5gm/kg and 1.5gm/kg body weight for 28 days as per limit test of OECD guideline. Group I was kept as normal control which received distilled water for 28 days, while in the IInd and IIIrd groups aqueous extract of the drug was administered orally at a dose of 0.5gm/kg and 1.5gm/kg body weight for 28 days. The animals were observed for gross behavior (salivation, lacrymation, lethargy, sleep and coma) and mortality at 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 24 hours and thereafter, once every day up to 28 days after drug administration. On 29th day blood was collected of all the three groups of rats for estimation of SGOT, SGPT and Serum alkaline Phosphatase,Serum urea and Serum Creatinine, Serum cholesterol, Serum triglyceride and Serum HDL,Percentage hemoglobin,ESR,Total leukocyte count and Differential leukocyte count. After collection of blood, the animals in all the three groups were sacrificed and liver, heart, kidney and spleen were excised out for determination of organ to body weight ratio. SGOT, SGPT were estimated by Reitman's and Frankel, 1957method (Reitman et al., 1957). Alkaline Phosphatase was estimated by Bessey and Brock, 1946 method (Bessay et al., 1946). Serum urea was estimated by GLDH, Ureas methodgiven by (Tiffany et al.,1972), while Serum Creatinine was estimated by Jaffe's methodgiven by (Bower'set al.,1980). Serum HDL was estimated by Phosphotungstic Acid method given by (Burstein et al.,. 1970), while Serum cholesterol and triglyceride were estimated by CHOD-PAP methodgiven by (Roeschlauet al.,1974) and GPO-Trindev methodgiven by (Mcgowan et al.,1983). ESR and DLC were estimated by Westergreen and Leishman stain methodsgiven in Medical Laboratory Technology (Mukherjee, 1990). TLC was estimated by Hemoaltometry method given by (Plum, 1936). Percent hemoglobin was estimated by Sahli's Acid Haematin method given by (Newcomer, 1919).

### Statistical Analysis

Statistical analysis was performed by using unpaired t-test calculating p-value at 5 % level. All values are expressed as Mean±SEM (standard error of mean). p-value less than 0.05 found to be considered statistically significant.

## Results and Discussion

### Hypoglycemic activity

The administration of aqueous extract of UNIM-215 at both the doses reduced significantly the alloxan induced elevation of blood glucose level for maximum treatment period as presented in table-1.

### Acute Toxicity Study

The effect of oral administration of single dose of aqueous extract of UNIM-215 in Swiss albino mice shows that the formulation was well tolerated by the animals and no abnormality was observed in the general behaviour of the animals and no overnight mortality was recorded. Herbs and supplements can be toxic when used for inappropriate indication, or prepared

inappropriately, or used in large excessive dosages or for a prolonged duration of time. Since it is polyherbal formulation other ingredients present in formulation helps in reducing the toxic effect of active component.

**Sub-Acute Toxicity Study**

The values of all the parameters including liver functions, renal functions,hematology and organ body weight ratio found to be normal as compared to control group. The effects of the studied drug on organ body weight ratio in control and treated animals are presented in Table-6. There were no significant changes observed in organ body weight ratio of the control and the animals treated with various doses. Table 2-4 is a summary of the results of the effects of the drug on the biochemical parameters. There were no significant changesin AST and ALT levels in all the treated animals compared with the control. Similarly serum urea, serum creatinine and lipid profiles of treated animal was found to be normal as compared to control group. Table-5 reflects the values for hematological parameters of treated group as compared to control showed no significant changes in Hb, TLC, ESR, and % lymphocyte except % polymorph count in group III animals where p-value = 0.333. This is not significant to affect safety of the drug. It can be concluded on the basis of above observation that drug is safe as it has not shown any toxic effects.

## Conclusion

The formulation has capacity to reduce alloxane induced hyperglycemia and was well tolerated by the animals. No abnormality was observed in the general behavior of the animals and no overnight mortality was recorded. There were no finding of any organ toxicity and hematological changes as laboratory findings were normal. It can be concluded on the basis of above observation that drug is efficacious and quite safe in animals.

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**Table-1 Antidiabetic activities of aqueous extract of UNIM-215 in alloxan induced Diabetic rat**

| Blood glucose Level in mg/dl | | | |
|---|---|---|---|
| Duration of days after treatment | | | |
| **GROUP ▾** | 1st day | 5th day | 10th day |
| **Group I Diabetic rats (n = 6)** | 187.72 ±5.41 | 177.86 ± 8.34 | 185. 60 ± 5.77 |
| **Group II Diabetic rats treated with glibenclamide 5mg/kg (n =6)** | 186.26±5.04 | 150.86 ± 5.29 ** | 148.85± 6.33 ** |
| **Group III Diabetic rats treated with 0.5gm/kg of drug extract (n = 6)** | 178.70 ± 8.08 | 168.19 ± 4.22 | 157.57 ± 5.93 * |
| **Group III Diabetic rats treated with1.5gm/kg of drug extract (n = 6)** | 183.78 ± 6.07 | 178.95 ± 7.51 | 165.88 ± 6.17 * |

Values are mean ± SEM
n = number of rats in a group.
**P* < 0.05, P** < 0.01 P*** < 0.001**

**Table-2 Effect of aqueous Extract of UNIM-215 on Liver function test in albino rats**

| PARAMETERS ► GROUP ▼ | SGOT U/L | SGPT U/L | SALP U/L |
|---|---|---|---|
| Group I Normal Control (n = 6) | 144.58 ± 7.0140 | 44.19 ± 2.68 | 105.24 ±11.24 |
| Group II Drug extract treated 0.5gm/kg (n = 6) | 136.30 ± 7.7307 | 42.35 ± 1.60 | 110.922 ± 10.38 |
| Group III Drug extract treated 1.5gm/kg (n =6) | 144.45 ±8.4433 | 42.72 ± 1.06 | 128.19 ± 16.87 |

Values are mean ± SEM
n = number of rats in a group.
**P* < 0.05, P** < 0.01 P*** < 0.001**

**Table-3 Effect aqueous Extract of UNIM-215 on Renal function test in albino rats**

| PARAMETERS ► GROUP ▼ | Serum Creatinine mg/dl | Serum Urea mg/dl |
|---|---|---|
| Group I Normal Control (n = 6) | 1.49 ± 0.041 | 50.49 ± 3.57 |
| GroupII Drugextracttreated 0.5g/kg (n = 6) | 1.55 ± 0.028 | 51.42 ± 2.93 |
| Group III Drug extract treated 1.5 gm/kg (n = 6) | 1.56 ± 0.015 | 43.59 ± 4.32 |

Values are mean ± SEM
n = number of rats in a group.
**P* < 0.05, P** < 0.01, P*** < 0.001**

**Table -4 Effect of aqueous Extract of UNIM-215 on Lipid Profile in albino rat**

| PARAMETERS ► GROUP ▼ | Serum Cholesterol mg/dl | Serum HDL mg/dl | Serum Triglyceride mg/dl |
|---|---|---|---|
| Group I Normal Control (n = 6) | 68.78 ± 2.58 | 23.43 ±0 .49 | 54.88 ± 5.21 |
| Group II Drug extracttreated 0.5gm/kg (n = 6) | 70.06 ±4.03 | 21.79 ± 1.76 | 59.87 ± 3.60 |
| Group III Drug extracttreated 1.5gm/kg (n = 6) | 64.45 ± 2.68 | 23.73 ± 1.41 | 52.42 ± 5.61 |

Values are mean ± SEM
n = number of rats in a group.
**P* < 0.05 P** < 0.01, P*** < 0.001**

**Table-5 Effect of aqueous Extract of UNIM-215 on Haematological studies in albino rats**

| PARAMETERS ► GROUP ▼ | Haemoglobin gm % | TLC/ Cumm | ESRmm /hr | DLC | |
|---|---|---|---|---|---|
| | | | | % Polymorph count | % Lymphocyte count |
| **Group I Normal Control (n = 6)** | 14.0±0.30 | 5533± 422.42 | 1.83±0.16 | 50.66±2.41 | 52.00± 1.91 |
| **Group II Drug extract treated 0.5gm/kg (n = 6)** | 14.40±0.26 | 5308±433.86 | 1.66 ±0.21 | 49.00± 1.26 | 51.83± 1.79 |
| **Group III Drug extract treated 1.5g/kg (n = 6)** | 14.26± 0.31 | 5400±381.88 | 2.0± 0.25 | 46.66± 3.11 | 52.66± 3.13 |

Values are mean ± SEM
n = number of rats in a group.
**P* < 0.05, P** < 0.01, P*** < 0.001**

**Table-6 Effect of aqueous Extract of UNIM-215 on Relative organ weight per 100gm body weight in albino rats**

| PARAMETERS ► GROUP ▼ | Organ weight in gm / 100 g body weight | | | |
|---|---|---|---|---|
| | Liver | Heart | Kidney | Spleen |
| **Group I Normal Control (n = 6)** | 3.99± 0.22 | 0.4200± 0.00097 | 0.9217± 0.0494 | 0.3633± 0.0191 |
| **Group II Drug extracttreated 0.5gm/kg (n = 6)** | 3.87± 0.19 | 0.4267± 0.0102 | 1.0117± 0.0491 | 0.395± 0.0141 |
| **Group III Drug extract treated 1.5gm/kg (n = 6)** | 4.20± 0.18 | 0.4183± 0.0347 | 0.9833± 0.0463 | 0.3617± 0.0168 |

Values are mean ± SEM
n = number of rats in a group.
**P* < 0.05, P** < 0.01, P*** < 0.001**

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# Evaluation of Paracetamol Tablets Using Carboxymethyl Starch as Disintegrating Agent

**Versha Parcha[1*], Alok maithaini[1], Babita Mahajan[2] and Mukesh Rawat[1]**
[1] SBSPGI of Biomedival sciences and Research, Balawak, Uttarakhand, India
[2] GGM Science College, Jammu, India
*E-mail: vershaparcha@gmail.com

**Abstract-** Starch is used as a common disintegrating agent in manufacturing of tablets because of its wide availability, economic and fast swelling property. However, the poor water solubility limits penetration of medium into the tablet which ultimately delays disintegration of tablets and slows down the onset of action. Thus, a fast disintegrating tablets can be used to minimize the onset of action of drug. Starch can be modified to increase its water solubility for better penetration of medium into the tablet resulting in rapid disintegration to get immediate action of the tablet. Carboxymethylation of starch is a widely studied conversion since it is simple and leads to products with not only improved water solubility and but high swelling index (bulk of CMS rise thirty times after swelling,) which can accelerate swelling of tablet and the dissolution of effective component and thus can be utilized as a disintegrating agent in manufacturing of tablet replacing starch. In view of this, the present study was taken up where starch extracted from maize was modified and converted to carboxymethyl derivative and was used as a disintegrating ingredient replacing starch in the paracetamol tablets . The tablets were evaluated not only for their disintegration profile but for all quality control parameters so as to come to conclusion that the replacing starch by CMS improves quality of paracetamol tablets. Results indicated that all the parameters were found to be within the prescribed limits in accordance to Indian Pharmacopeia. In specific disintegration test, the results revealed that the effect of Super disintegration agents, C.M.S. significantly reduced the disintegration time of prepared tablets which helps to get immediate action of the tablet. However, detailed studies are required to confirm the above said action using different ratios of CMS with different kinds of tablets.

**Keywords**: Carboxymethylation, Paracetamol, Disintegrating agents

## Introduction

Polysaccharides are made of many or more than ten monosaccharide units joined together by glycosidic linkage. The chemical modification of polysaccharides is the most important route to modify the properties of the naturally occurring biopolymers and to use this renewable resource in the context of sustainable development.. However, up to now only a limited number of products are produced commercially.

Starch stored mainly in seeds, roots and tubers constitute the reserve food material of plant and is utilized for metabolic uses by the enzymatic breakdown..Also it is used as a source of energy to body, as sizing material in paper industry, finishing agent in textile industry, in laundries for stiffening clothes and for manufacture of glucose, alcohol, dextrin, explosive, etc. Chemically, starch is composed of two different molecules, amylose and amylopectin. In amylose, the glucose molecules are linked in a "linear" fashion whereas amylopectin is branched polymer of glucose. Starches are modified to enhance their performance and can have wide applications. They may also be modified to increase their stability against excessive heat, acid, shear, time, cooling, or freezing; to change their texture; to decrease or increase their viscosity; to lengthen or shorten gelatinization time; or to increase their viscous stability[1-3]Carboxymethylation of polysaccharides (starch) is a widely studied conversion since it is simple and leads to products with a variety of promising properties. One of the most important goals of carboxymethylation of starch is to obtain water-soluble derivatives which are applied in various fields in order to control the behavior of aqueous systems and

preparations.CMS can be used as a swelling agent since the bulk of CMS rise thirty times after swelling, which can accelerate swelling of tablet and the dissolution of effective component and thus can be utilized as a disintegrating agent in manufacturing of tablet.

In view of this in the present study, starch was extracted from maize, derivetized and used as a disintegrating ingredient for paracetamol tablets and was evaluated for the same.

## Material and Methods

### Extraction and Derivetization of starch from Maize:

Maize (1kg) was collected from local market of Dehradun, washed and macerated with water. The starch was extracted by usual method. Starch so obtained (10gms) was suspended in 100 ml 2-propanol, 45% NaOH solution was added to it and then stirred at 30°c for 10 minutes. 30 gms monochloroacetic acid and the solution was further added and the solution so obtained was stirred for next 30 minutes. pH of the solution was maintained at 5.0 with 50% glacieal acitic acid. The product so obtained was purified by washing with 95% methanol about 10 times and kept it in desiccators for 10 days.The dried CMS was placed in hot air oven at 50°c for 10 hours before passing through 80 mess sieves and then was used in the preparation of paracetamol tablet replacing conventional disintegrating agent by CMS.

### Preparation of Paracetamol Tablet Using CMS

[3]Paracetamol tablet was prepared by wet granulation method using Paracetamol (80%) as active pharmaceutical ingredient, lactose (10%) as a bulking agent, sucrose as binder (2%), magnesium stearate as lubricant (1%), CMS as disintegrating agents(6%), talc as antiadhersant and glidant (5%).

### Evaluation of Paracetamol Tablet [4-8]

### Weight Variation

Weight variation was carried out to ensure that each of the tablets contain the proper amount of drug. The test was carried out by weighing 20 tablets individually using analytical balance, then calculating the average weight and comparing the individual tablet weights to the average. The percentage of weight variation is calculated by

$$\% \text{ weight variation} = \frac{\text{individual w.t} - \text{average w.t X100}}{\text{Average w.t}}$$

### Tablet Thickness

The thickness of the tablets was measured by Vernier caliper by placing them between two arms of the Vernier caliper.

### Hardness Test

The resistance of tablets to capping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness. Tablet hardness is defined as the load required for crushing or fracturing a tablet placed on its edge. Sometimes, it is also termed as tablet crushing strength. The hardness test was performed using Monsant type (Make: Singhla) hardness tester. The instrument measures the force required to break the tablet when the force is generated by anvils to the tablet. The tablet was placed between two anvils; force applied to the anvils, and the crushing strength that just causes the tablet to break was recorded. The crushing strength test was performed on 20 tablets from each formulation.

### Friability

For each formulation, the friability of 20 tablets was determined using Roche type friabilator. 20 tablets from each formulation were weighed and tested at a speed of 25 rpm for 4 mins. After removing of dusts, tablets were re-weighed and friability percentage was calculated using the following equation.

$$\% \text{ friability} = \frac{\text{weight of tab before friability} - \text{weight after friability X 100}}{\text{Wt of tablet after friability}}$$

### Disintegration

The disintegration apparatus described in I.P was used for the study. It contains 2 basket rack assembly. Each basket rack assembly consists of 6 glass tubes that are 3 inches long, open at the top and held against 10 mesh screen at the bottom.

Each tablet was placed in each tube, and the basket rack was positioned in 1-L beaker of distilled water. Temperature at 37±2°C was maintained throughout the study.

**Dissolution**

The *in-vitro* dissolution study of tablets for best formulation was determined by using USP Type 2 (paddle type) dissolution apparatus. The test was performed in 200 ml of distilled water at 50 rpm maintained at 37± 0.5°C. The specific amount of samples were withdrawn at predetermined time intervals for period of 2 hours (15, 30, 45, 60, 90, 120 mins.) and replaced with the equal volume of the same dissolution medium. The samples were filtered through 0.2µm membrane filter.

## Results and Discussion

Starch is used as a common disintegrating agent in manufacturing of tablets because of its wide availability, economic and fast swelling property. However, the poor water solubility limits penetration of medium into the tablet which ultimately delays disintegration of tablets and slows down the onset of action. Thus fast disintegrating tablets can be used to minimize the onset of action of drug. Rapid disintegration can be achieved by using superior disintegrating agents. The starch which is partially soluble in water, on modification gets freely soluble in water. Therefore, in our present study starch was derivetized as carboxymethyl derivative(CMS) with increased water solubility and improved swelling index and was thus used as disintegrating agent in paracetamol tablet replacing starch. Paracetamol tablets so prepared were evaluated for various quality control parameters. Results of the study are summarized in the tables(1 to 5) and discussed at appropriate places.

**Table-1 Weight variation test of paracetamol table (Average weight 488 mg)**

| S.no | Individual weight of tab (mg) | %weight variation | S.N | Individual weight of tab (mg) | %weight variation |
|---|---|---|---|---|---|
| 1. | 490 | 0.40 | 15 | 490 | 0.40 |
| 2. | 470 | -3.6 | 16 | 490 | 0.40 |
| 3. | 510 | 4.5 | 17 | 490 | 0.40 |
| 4. | 490 | 0.40 | 18 | 490 | 0.40 |
| 5. | 450 | -7.7 | 19 | 490 | 0.40 |
| 6. | 500 | 2.4 | 20 | 500 | 2.4 |
| 7. | 490 | 0.40 | 21 | 480 | -1.6 |
| 8. | 480 | -1.6 | 22 | 480 | -1.6 |
| 9. | 490 | 0.40 | 23 | 490 | 0.40 |
| 10. | 490 | 0.40 | 24 | 490 | 0.40 |
| 11. | 490 | 0.40 | 25 | 490 | 0.40 |
| 12. | 490 | 0.40 | | | |
| 13. | 500 | 2.4 | | | |
| 14. | 490 | 0.40 | | | |

The maximum weight variation obtained was ± 0.40%, which falls within the acceptable weight variation range of ± 5%. Hence all the tablets passed the weight variation test. According I.P limit of weight variation of various tabs is 130 or less- ± 10%, 130-350- ± 7.5%, <350- ±5%

**Table-2 Thickness of paracetamol tablet.**

| S.no | Thickness of test | Thickness of standard |
|---|---|---|
| 1. | 3.7mm | 3.8mm |
| 2. | 3.8mm | 3.8mm |
| 3 | 3.8mm | 3.8mm |
| 4. | 3.7mm | 3.8mm |
| 5 | 3.8mm | 3.8mm |

The thickness test of the table was 3.7 and the std. tablet thickness was 3.8 .So we know that our formulation compiles to each other

**Table-3 Hardness of paracetamol tablet**

| S.no. | Hardness of test | Hardness of standard |
|---|---|---|
| 1. | 3.10±0.08 | 4 kg/cm$^2$ |
| 2. | 4.03±0.14 | 4 kg/cm$^2$ |
| 3. | 4.21±0.09 | 4 kg/cm$^2$ |
| 4. | 4.06±0.11 | 4 kg/cm$^2$ |
| 5. | 4.13±0.12 | 4 kg/cm$^2$ |

According to I.P the limit of hardness is 4 to 9. Hardness for tablets was in the range of 4.0 to 4.2 kg/cm2, which falls above the limit of not less than 3.0 kg/cm2.

**Table-4 Friability value of paracetamol tablet**

| S.no. | Initial w.t of 25 tab (gm) | Final wt of 25 tab (gm) | % friability for test |
|---|---|---|---|
| Test | 8.705 | 8.631 | 0.85 % |
| Standard | 8.706 | 8.621 | 0.98% |

None of the tablets showed friability value more than 0.85% ,which is less than ideal limit 1%.

**Table -5 Disintegration time of paracetamol tablet**

| S.no | Standard tablet | Disintegration time | Test tablet | Disintegration time |
|---|---|---|---|---|
| 1. | PCM-1 | 14 | PCM-1 | 09 |
| 2. | PCM-2 | 15 | PCM-2 | 09 |
| 3. | PCM-3 | 15 | PCM-3 | 09 |
| 4. | PCM-4 | 14 | PCM-4 | 07 |
| 5. | PCM-5 | 14 | PCM-5 | 08 |

All the tablets disintegration time ranges within the limit suggested by IP for uncoated tablets i.e., NMT 15 minutes. However, the disintegration time of test tablet (CMS) is superior to that of the standard paracetamol tablet (starch).

**Table-6 Dissolution value of paracetamol tablet**

| Sampling Time (Min) | Basic medium (7.4 pH) | | Formula | % of release |
|---|---|---|---|---|
| | Direct absorbance | Y= mx+c | (µg/ml X 900)/100 | |
| 15 | 0.285 | 0.285= 0.0426x- 0.1878 $X = \frac{0.285 - 0.1878}{0.0426}$ | 2.28*900/100 | 20.5% |
| 30 | 0.380 | 0.380= 0.0426x- 0.1878 $X = \frac{0.380 - 0.1878}{0.0426}$ | 4.511*900/100 | 45.6% |
| 45 | 0.480 | 0.480= 0.0426x- 0.1878 $X = \frac{0.480 - 0.1878}{0.0426}$ | 6.85*900/100 | 67.7% |
| 60 | 0.585 | 0.585= 0.0426x- 0.1878 $X = \frac{0.585 - 0.1878}{0.0426}$ | 9.32*900/100 | 90.9% |
| 90 | 0.650 | 0.650= 0.0426x- 0.1878 $X = \frac{0.650 - 0.1878}{0.0426}$ | 10.84*900/100 | 98.6% |

Dissolution studies were performed to evaluate the drug release profile of tablet which was observed to be ranging in the prescribed limit (IP)

## Conclusion

From the above study, it could be concluded that in the prepared paracetamol tablets with CMS as disintegrating ingredient, all the evaluation parameters were found to be within the prescribed limits in accordance to Indian Pharmacopeia and in the specific disintegration, time was significantly reduced which enhances immediate action of the tablet. However, detailed studies are required to confirm the above said actions using different ratios of CMS with different kinds of tablets.

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# Evaluation of Better Antimicrobial Activities Amongst *Sapium sebiferum* and *Artocarpus heterophyllus*

**Suman Rawat[1]*, O.P.Sati[2], Uday kumar[3]and Ankita sati[4]**

[1, 3, 4] Department of Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India.

[2]Department of Chemistry, H.N.B.Garhwal Central University, Uttarakhand, India.

*E-mail: sumanman.2008@rediffmail.com

**Abstract-** The present study was carried out to evaluate the comparative antimicrobial activities of *Sapium sebiferum* and *Artocarpus heterophyllus*. Phytochemical screening of the *S.sebiferum* and *A.heterophyllus* showed the confirmation of carbohydrates, phenolic compounds and tannins. The comparative assessment of antifungal activity was performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar) against *Aspergillus niger*. The antibacterial effect was studied by the agar direct contact method using *Bacillus cereus* and *E.coli*. The results revealed that the acetonic extract of *Artocarpus heterophyllus* showed good antifungal activity against *Aspergillus niger* in comparison to the standard drug (Clotrimazole), while acetonic extract of *Sapium sebiferum* showed no antifungal activity. Acetonic extract of *Sapium sebiferum* showed good antibacterial activity against *B.cereus* (25mm) and *E.coli* (20 mm) inhibition zone. Whereas Acetonic extract of *Artocarpus heterophyllus* showed less antibacterial activity i.e. zone of inhibition against *B.cereus* (10mm) and *E.coli* (9 mm). Both plant extracts have high medicinal value. These results suggest that traditional folk medicine could be used as a guide for search for new natural products with potential medicinal properties.

**Keywords**: *Sapium sebiferum*, *Artocarpus heterophyllus*, Antimicrobial activity, Phytochemical studies.

## Introduction

***Sapium sebiferum Roxb*** *is* a plant belonging to family ***Euphorbiaceae.***[1]It is monoeciouces, deciduous small tree upto 13 m tall, stem often gnarled bark whitish grey with vertical cracks, containing white latex. Leaves are alternate, broad rhombic to ovate in shape 3.8-8.5 cm long and have smooth edges, heart shaped .Its wood has been used to make various implements,toys, furniturs inferior quality pencils, cricket bats and Chinese printing blocks.[2] Resin from root bark is considered as purgative. The seed is antidote, emetic and purgative.[3]

***Artocarpus heterophyllus*** *is* a plant belonging to family ***Moraceae***It is a large,evergreen tree ,10-15 m in height, stem is straight rugh whereas bark is green or black,1.25cm thick, exuding milky latex[4]. Leaves are dark green, alternate, simple, glossy, large and elliptic to oval in form. Fruits are oblong cylindrical in shape. Its leaves are useful in fever, boils, wounds and skin diseases. The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent. The root is a remedy for skin diseases and asthma. The wood has a sedative property. Latex is used as an anti-inflammatory agent[5]

## Material and Methods

### Collection and Identification of Leaves of *Sapium sebiferum Roxb* and *Artocarpus heterophyllus*

Leaves of *Sapium Sebiferum Roxb* and *Artocarpus heterophyllus* were collected from FRI, Dehradun and Manduwala Chakrata road,Dehradun, India resepectively. Plant material was authenticated by S. K. Srivastava (Scientist D/HOD, Botanical

Survey of India, Northern regional centre, Dehradun).

**Extraction of leaves of *Sapium sebiferum Roxb* and *Artocarpus heterophyllus* in different solvents (Non-polar to Polar)**

The collected plant material was washesd with water to remove other undesirable material and dried under shade. The air-dried leaves (200 gm) of both were crushed. The crushed leaves extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone and methanol by hot percolation method using Soxlet apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure and used for antimicrobial activity.

**Anti-microbial activity of different extracts**

The **anti-microbial activity** of the leaves of ***Sapium sebiferum Roxb and Artocarpus heterophyllus*** was carried out. The leaves extract were screened for antibacterial and antifungal activities.

## Antibacterial activity of leaves extract

In this study, the anti bacterial activity was studied against micro organism and bacterial cultures used in the study were: *B.cerus, B.pumilus, M.luteus, Escherichia coli*

These bacterial cultures were maintained on nutrient agar slants at first being incubated at $37^0$ c for about 18-24 hours and then stored at $4^0$ c as stock for anti bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at $37^0$c overnight. To test anti bacterial activity, the well diffusion method used.

**Culture Media Preparation**

The microbiological media was prepared as per standard instructions provided by HI-Media Laboratories, Mumbai. The media used for anti-bacterial activity were Muller- Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at $121^0$C at 15 psi for 15-30 minutes in autoclave.

**Plate Preparations**

25 ml of pre autoclaved Muller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri-plates. These petri-plates were allowed to solidify at room temperature.

**Well Diffusion Method**

After the plates solidified, the freshly prepared microbial growth culture suspension (about 20µl) was spread over the Muller – Hinton agar (MHA) media using L shaped sterilized glass spreader separately under the aseptic condition using laminar air flow. Then well were made in each plate with the help of borer of 8 mm diameter .In these well, about 100µl of each leaf-extract individually was loaded. This method depends upon the diffusion of leaves extracts from hole through the solidified agar layer of petri-dish to such an extent that the growth of added micro organism is prevented entirely in a circular area or Zone around the hole containing leaf extract.

**Incubation:**

Petri plates were incubated for overnight at $37^0$C ± $0.5^0$C.

**Measurement of Zone of Inhibition**

After incubation, the diameter of clear zone of incubation produced around the well or holes were measured in mm by ESR Tube and compared with the standard drug.

# Results

**Table-1 Antibacterial activity of different extracts of *Sapium sebiferum Roxb* and standard drug chloramphenicol**

| S. No. | Test organism | Inhibition zone in mm | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pet.Ether | Chloroform | Acetone | Methanol | Standard drug | | |
| | | | | | | Ampicilline | Streptomycin | Chloram-phenicol |
| 1 | E. coli | 10 | 20 mm | 20 | - | 20mm | 17mm | 25mm |
| 2 | Bacillus cereus | 18 | 16 mm | 25 | - | 15mm | 16mm | 36mm |
| 3 | B.pumilus | - | 20 | 25 | 22 | _ | 16mm | 20mm |
| 4 | M.luteus | - | 10 | 14 | 16 | 30mm | 26 mm | 30 mm |

**Table-2 Antifungal activity of different extract *Sapium sebiferum Roxb* and standard drug Clotrimazole.**

| S. No. | Test Organism | Inhibition zone in mm | | | | |
|---|---|---|---|---|---|---|
| | | Pet. Ether | Chloroform | Acetone | Methanol | Standard drug Clotrimazole |
| 1 | *Aspergillus niger* | _ | _ | - | - | 11mm |
| *2* | *M.gypseum* | 18 | 14 mm | 20 mm | - | _ |
| 3 | T. flavurusclem | 12 mm | 12 mm | 22 mm | - | 12mm |

**Table-3 Antibacterial activity of different extracts of *Artocarpus heterophyllus* and standard drugs**

| S. No. | Test organism | | Inhibition zone in mm | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pet.Ether | Chloroform | Acetone | Methanol | Standard drug | | |
| | | | | | | Ampicilline | Streptomycin | Chloram-phenicol |
| 1 | *E. coli* | 1 | - | 9 | 19 | 20mm | 18mm | 20mm |
| 2 | *Pseudomonas aeruginosa* | 4 | 7 | 16 | 17 | - | 18mm | 18mm |
| 3 | *Bacillus cereus* | 8 | 10 | 10 | 14 | 16 | 17mm | 18mm |
| 4. | *M.aureus* | - | - | 7 | 8 | 24 mm | 27 mm | 15 mm |

**Table-4 Antifungal activity of different extract *Artocarpus heterophyllus* and standard drug Clotrimazole.**

| S. No. | Test Organism | | Inhibition zone in mm | | | | |
|---|---|---|---|---|---|---|---|
| | | Pet. Ether | Chloroform | Acetone | Methanol | Standard drug | |
| | | | | | | Amphotericin-B | Clotrimaz ole |
| 1 | *Aspergillus niger* | 13 | _ | 29 | 11 | _ | 19 mm |
| *2* | *Candida albicans* | 9 | 12 | 13 | 13 | 13 | 11 |
| 3 | *Sclorotium* | 6 mm | - | 13 | 7 | _ | - |
| 4 | *Rhizopus* | 9 | - | 16 | 11 | - | - |
| 5 | *Microsporum* | 6 | 4 | 18 | 13 | - | - |

## Discussion

The antibacterial activity of leaves extracts of ***Sapium sebiferum***was found active against *E.coli*, *Bacillus cereus*, *B.pumilus* and *M.luteus* in chloroform and acetone extracts whereas leaves extracts of Petroleum ether was found inactive against *B.pumilus* and *M.luteus.* The antifungal activity of leaves extracts of acetone was found highly active against *M.gypseum* and *T. flavuruscleum* while inactive against *Aspergillus* niger. The results revealed that the **Acetonic extract of *Sapium sebiferum*** has shown more degree of antimicrobial activity than other extract when compared to the standard drug. It is due to presence of chemical constituents like alkaloids, proteins, carbohydrates, phenolic compounds, tannins, amino acid, fats and fixed oil and saponins which was confirmed by phytochemical

studies. While in case of ***Artocarpus heterophyllus*** the **methanolic extract** showed good antibacterial activity against *B.cereus* and *Pseudomonas aeruginosa* and the acetonic extract showed good anti-fungal activity against *Rhizopus , Microsporum* and *Aspergillus niger* in comparison to the standard drug.

## Acknowledgment

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# Antifungal Potential in the Juice Extracts of Selected Herbal Plants: Turmeric, Garlic, Ginger and Onion

**Ajay Singh* and Vipin Panwar**
Department of Chemistry, Uttaranchal University, Dehradun, Uttarakhand, India, 248006.
*E-mail: ajay21singh@yahoo.com and hodchemistry@uttaranchaluniversity.ac.in

**Abstract-** Herbal plants produce and contain a variety of chemical substances which can have pharmacological activities .They can defend against attack from predators such as insects, fungi and herbivorous mammals. The *in vitro* inhibitory effects of juice extracts of the selected herbal plants were studied against selected fungi. Selected four fungi are *A. niger, A. terreus, A.japinicus and P. expansum*. Fungi are strongly inhibited by the garlic juice than turmeric and ginger. Onion juice extracts have shown minimum inhibition. In comparision to reference antifungal agent (Ketacanazole), garlic and ginger have showed effective inhibition against selected fungi. Combination of these juice extracts also have shown antifungal activity.

**Keywords:** Herb, *Allium cepa*, *Allium sativum*, Extract, Fungi.

## Introduction

Herbal plants produce and contain a variety of chemical substances which can have pharmacological activities . They can defend against attack from predators such as insects, fungi and herbivorous mammals .Herbal plants are being used for curing many diseses since ancient time. The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. The phytochemicals in them have beneficial health effects when they are consumed by humans for longer time and they can be used to effectively treat human diseases (Lai PK, Roy J; Roy. June 2004). It is the secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs. For example, quinine from the cinchona, morphine and codeine from the poppy are used in making drugs. (Tapsell LC, Hemphill I, Cobiac L. et al., August 2006). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. (Cai, Yun; Wang, Rui; Pei, Fei; Liang, Bei-Bei (2007).

Natural products once used to serve as the only source of medicine for the mankind in the ancient time. Many plants used today were known to people of ancient culture throughout the world. They valued their preservative and medicinal powers. (Lawson L.D., Abrams G; 1998). Naturally occurring microbial inhibitors have been recovered from a wide variety of foods including onions, garlic, fruits, vegetables, cereals and spices. many of these antimicrobials contribute to the food stuffs, natural resistance to deteriorations. The flavour components consist of such compounds as alcohols, aldehydes, esters, terpens, phenols, organic acids and others, some of which have not yet been identified. The widespread use of garlic and onion as a flavoring agent is well known. Garlic bulbs contain pectin, garlicin, volatile oils, allin and allistatins I and II. Alcoholic extract of garlic shows bactericidal, antibiotic, high hypoglycemic and fungicidal activities (Tessema, B; Mulu, A; Kassu, A; Yismaw, G (2006). The extract showed hypotensive, analgesic sedative and antileptazol properties. Onion and garlic also known to have medicinal properties. Onion bulbs contain tannin,

pectin, quercetin and glycosides. Spice extractives, such as of garlic shows bactericidal, antibiotic, high hypoglycemic and fungicidal activities. The extract showed hypotensive, analgesic sedative and antileptazol properties. Onion and garlic also known to have medicinal properties, oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavor in some products. Alcohol extracts show hypotensive analgesic and antileptazol properties. Onion extracts shows antibacterial properties. (Lawson L. D, Abrams G; 1998).

**Herbal Plants Selected**

**Garlic (*Allium sativum*):** Garlic has been used extensively in herbal medicine (phytotherapy, sometimes spelt phitotherapy). Raw garlic is used by some to treat the symptoms of acne and there is some evidence that it can assist in managing high cholesterol levels. It can even be effective as a natural mosquito repellent. (Amagase H., Milner J., 1993) Important constituents are allicin, diallyltrisulphide, and alliin.

**Onion (*Allium cepa*):** The constituents of onion contain only traces (0.01%) of essential oil, which mostly consists of sulfur compounds. Onions contain two substances: sulfur and quercetin - both being strong antioxidants. They each have been shown to help neutralize the free radicals in the body, and protect the membranes of the body's cells from damage.It also contains the minerals Potassium , Phosphorus, Calcium ,Magnesium, Sodium and Selenium (Jandke J. *et al.* 1987)

**Ginger (*Zingiber*):** The characteristic odor and flavor of ginger is caused by a mixture compounds of zingerone shogaols and gingerols which are volatile oils that compose one to three percent of the weight of fresh ginger. Gingerols can inhibit growth of ovarian cancer cells. *Wood, C. (1988).*

**Turmeric (*Curcuma longa*):** Turmeric is a rhizomatous herbaceous plant of the ginger family, Zingiberaceae The most important chemical components of turmeric are a group of compounds called curcuminoids which include curcumin (diferuloylmethane), demethoxycurcumin and bisdemethoxycurcumin. The best-studied compound is curcumin, which constitutes 3.14% (on average) of powdered turmeric**.** (Tayyem R.F. et al. 2006) In addition, other important volatile oils include turmerone, atlantone, and zingiberene. Some general constituents are sugars, proteins, and resin. (Mishra S, 2008.)

## Material and Methods

**Sampling**

About 500g of each plant samples were taken for this study. Collection of plant samples (garlic, onion, Ginger, turmeric and lemon) was done from the mandi market of Dehradun, Uttarakhand, India.

**Preservation of samples:** The samples were kept in refrigerator at 4º C.

**Preparation of plant juice extracts for antimicrobial activity**

At first, skins of the garlic,turmeric, ginger and the onion bulbs were peeled out and washed with sterilized water and air dried for 1 hour and cut in small pieces. Then the garlic and onion pieces were grinded in electric blender separately. Using the clean and dry muslin cloths, the crude juices was squeezed out, then it was further filtered through the whatmann filter paper No.1 under vacuum pressure. The filtrate was taken as for the experimental juice extracts sample.

**Combination of Juice extracts:** The extracted juices of garlic, ginger, turmeric, onion and lemon are mixed either of two samples (garlic and onion, garlic and lemon, onion and lemon) or of all three (garlic and onion and lemon) in the 1:1 and 1:1:1 ratio respectively.

**Preparation of antifungal solutions**
The stock solution of 10mg/ml was made first and further diluted to make concentration 1mg/ml using sterile distilled water.

**Antifungal activity of plant sample juices**
Fungal infections are the most common among the human population and a number of therapeutic agents are also available in the market but most of them are effective as topical applications. Rare drugs are available for deep mucosal infection.

In this study, the antifungal activity was studied against following cultures.
- *Aspergillus niger (MTCC 2479)*
- *Aspergillus terreus (MTCC 279)*
- *Aspergillus japinicus (MTCC 1975)*
- *Penicillium expansum (MTCC 2006)*

The cultures were obtained from the standard cultures maintained in the Microbiology department of Uttaranchal University, Dehradun, India. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25º C for about 72 – 96 hours and then stored at 4º C as stock cultures for further antibacterial activity. Fresh cultures were obtained by transferring a loopful of culture into Sabouraud Dextrose broth (SDB) and then incubated at 25º C for 72 hours. To test antifungal acivity, the Cup well diffusion method was used.

**Culture Media Preparation**
The microbiological media were prepared as standard instruction provided by the HI-MEDIA Laboratories PVT. Limited, Mumbai. The medium used for antifungal activity were SDA, SDB were prepared and sterilized at 121º C at 15 lbs for 15 – 30 minutes in autoclave.

**Plate Preparation**
About 25ml to 30ml of pre autoclaved SDA was poured into 90mm diameter pre sterilized petriplates and was allowed to solidify at room temperature.

**Cup or Hole Well Diffusion Plate Method**
After the plates solidified the freshly prepared 72hrs fungal broth culture suspensions about 0.1ml was spreaded over the SDA media using L-shaped sterilized glass spreader separately under aseptic condition using Laminar air flow.

Then four wells were made in each plate with the help of borer of 8mm diameter. In these well, about 0.1ml of each plant sample juice extracts were loaded and the antifungal drug solutions 1mg/ml (0.1ml) were also loaded in the wells as reference.

**Incubation**
The Petri plates were dark for 72 hours at 25º C in the incubator.

**Measurement of Zone of Inhibition**
After the incubation, the diameter of clear zone of inhibition produced around the well (or hole) were measured in mm and the diameter of inhibition by the juices extracts were compared with the reference antifungal agent (Ketacanazole).

## Results and Discussion

**Antifungal activity**

The antifungal agent taken as reference was - Ketacanazole.

**Garlic** juice extracts produced the highest zone of inhibition of *A. terreus* (45.33±0.47 mm) followed by *P. expansum* (22±0.81 mm), The order of fungi which were inhibited more strongly by the garlic juice extracts follows as: *A. terreus> P. expansum> A. niger> A. japinicus Turmeric and Ginger extracts also produced the highest zone of inhibition against A. terreus. (Table-1)*

The first published evidence by Schmidt and Marquardt, 1936 demonstrated the extraordinary fungistatic and fungicidal action of freshly pressed garlic juice and dried garlic with epidermophyte cultures. In **1960**, several workers carried out some model experiments with various yeast strains (*Saccharomyces cerevisiae, S.ellipsoideus, S.carlsbergensis*) and enzymatically-produced allicin. Effectiveness against *Aspergillus parasiticus, Aspergillus ochraceus, Penicillum patulum, P.roqueforti*, and *P.citrinum* has also been reported. *Saccharomyces cerevisiae, Candida albicans, Microsporum*

*canis, Trichophyton mentagrophytes*, and *T.rubrum* are further species responding to garlic. (Hughes and Lawson, 1991). Onion juice extracts could not produce the significant zone of inhibition against any fungal strains. (Table-1)

Shelef, L.A; 1983, *suggested that* onion has inhibitory effect against *Aspergillus flavis* and *Aspergillus parasiticus in higher concentration. However, all selected fungi were found to be resistant to the onion, it might be due to less concentration of juice applied.*In contrast to garlic, fungi are less sensitive to lemon, although the lemon had higher inhibitory effects against *A. terreus* than the effect shown by the ketacanazole.

**Antifungal activities of the plant juice extracts (applied in combination)**

The combination of **garlic and turmeric** juice **(GT)** produced zone of inhibition only against *A. japinicus* .This combination was found to be more potent against *A. japinicus* than the ketacanazole. Other fungi were not inhibited by this combination. The combination of **garlic** and **ginger (GGi)** is also effective against some fungi like *P. expansum. (Table-2)*

**Table-1 Mean Diameter (mm) and SD of zone of inhibition produced by the plant juice extracts (applied singly) against the fungi**

| Name of fungi | G | O | T | Gi | K | Most potent plant juice extracts |
|---|---|---|---|---|---|---|
| *A. niger* | 20.66±0.47 | ND | 21.16±0.11 | ND | 16.5±0.5 | G (20.66±0.47mm) |
| *A. terreus* | 45.33±0.47 | ND | 43.1±0.27 | 18.2±0.47 | 13.33±0.47 | G (45.33±0.47mm) |
| *A.japinicus* | 16±0.0 | ND | 14±1.0 | ND | 14.33±0.47 | G (16±0.0mm) |
| *P. expa nsum* | 22±0.81 | ND | 23±0.1 | 16.66±0.2 | 22.66±0.94 | G (22±0.81mm) |
| **Most Sensitive Fungi** | *A.t*(45.33±0.47 mm) | ND | *A.t*(43.1±0.27 mm) | *A.t* (18.2±0.47m m) | *P.e* (22.66±0.94 mm) | Most sensitive fungi *A. terreus* and potent plant juice **G** |

G= Garlic, O= Onion, T= Turmeric, Gi= Ginger K= Ketacanazole **(reference)**, ND= Not detected,
*At= A. terreus, Pe= P. expansum*

**Table-2 Mean Diameter (mm) and SD of zone of inhibition produced by plant juice extracts (in combination) against the fungi**

| Name of fungi | GT | GGi | GO | OT | K | Most potent plant juice combination |
|---|---|---|---|---|---|---|
| *A. niger* | 16.2±0.5 | ND | ND | ND | 18.5±0.3 | ND |
| *A. terreus* | ND | ND | ND | ND | 14.31±0.21 | ND |
| *A. japinicus* | 15.5 ±0.5 | ND | ND | 10.5 | 14.83±0.11 | GT |
| *P. expansum* | ND | 18.24 ±0.24 | ND | ND | 22.60 ±0.10 | GL (18.66± 0.94 mm) |
| **Most Sensitive Fungi** | *Aj* ±0.47mm) | *Pe* ±0.94mm) | ND | ND | *Pe* ±0.24mm) | Most sensitive fungi *P. expansum* and |

*Aj= A. japinicus, Pe= P. expansum*

**Antifungal Activity**

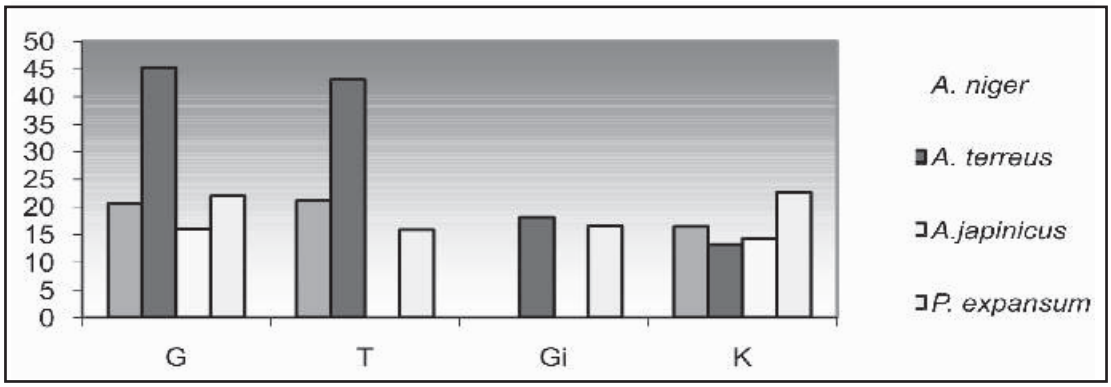


**Figure-Plant juice extracts (applied singly) against the selected fungi.**

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# Isolation and Yield Percentage of Different Fractions in Different Stages of *Diospyros peregrina*

**Deepika Chauhan[1*] and P. K. Gupta[2]**
[1]Cellulose and Paper Division
Forest Research Institute, Dehradun - 248006, India
[2]Uttaranchal College of Science and Technology, Sahastradhara Road, Dehradun-248001
*E-mail: cdeepika07@yahoo.com

**Abstract-** *Diospyros peregrina,* Gurke, an evergreen tree upto 15 m high grows all over India especially in the plains of coastal regions and river banks in India. Unripe fruits are astringent, acrid, bitter and oleaginous. They are used for the treatment of diarrhoea, dysentery, cholera, ulcer of mouth, and in wounds. Mature fruits are highly nutritious and contribute to household food security of rural people. *Diospyros peregrina* is a large genus of shrubs and trees comprising of 500 species distributed in the warmer regions. It belongs to the family, Ebenaceae. About 41 species occur in India mostly on evergreen forests of Deccan, Assam, and Bengal; only few are found in North India. The fruits fall to ground from June to July onwards and, under favourable conditions, the seeds germinate during rainy season. The aim of the present study focuses on isolation and yield percentage of pectic fractions at different stages of fruits of *Diospyros peregrina*. The main component of pectin is present as chelator soluble and carbonate soluble pectin and the majorly as uronic acid. Chelator soluble pectic fraction from AIS (Acetone Insoluble solid) in four stages of fruit ripening was maximum, 1.21±0.09% in colour initiation stage and minimum 0.56±0.04% in immature green stage. It was 1.03±0.12% and 0.85±0.05% in mature green stage and fully ripe stage respectively. The carbonate soluble pectic fraction was maximum, 9.25±0.34% in mature green stage and was minimum, 3.18±0.07% in fully ripe stage. It was 7.21±0.26% and 8.64±0.38% in immature green stage and colour initiation stage respectively. With fruit ripening, decrease in the amount of pectic substances was observed. Chelator-soluble pectins fraction and carbonate soluble pectin fraction undergo drastic degradation during ripening.

**Keywords:** Ebenaceae, *Diospyros peregrina*, Chelator soluble pectic fraction, Carbonate soluble pectin fraction.

## Introduction

Pectic substances, commonly known as pectins, are basically complex mixtures of polysaccharides whose major component consists of α-D-galacturonic acid units linked by α- (1 → 4) glycosidic bonds. In the main chain L-rhamnose occasionally inserted through (1 → 4) and (2 → 1) glucosidic linkages and the carboxyl groups are partially esterified by methyl alcohol or neutralized with mono or divalent cations. Other neutral sugars such as arabinose, ribose, galactose, glucose, mannose and xylose may occur attached as side-chains. The main sources of commercial pectin are citrus peel (lemon, lime and grapefruit), apple pomace and sugar beet pulps. Pectins from different sources are widely used as gelling agents, thickeners, texturisers, emulsifiers and stabilisers in food, pharmaceutical, and many others industries.

This multifunctionality of pectin is due to their functional groups which can be enhanced or even changed by introducing new functional groups onto the pectin polymer backbone using either chemical or enzymatic methods (Pappas et al., 2004). Chemical modifications of pectin can lead to new products with significant physicochemical and biological properties.

An introduction of non-polar residues increases hydrophobic character of pectin macromolecules. The hydrophilicity–lipophilicity relationship of such polymers depends on the degree of substitution, i.e. the content of non-polar substituents attached to the original polar macromolecule (Synytsya et al., 2004). At small degrees of substitution, the polymer is soluble in

water like the hydrophilic precursor. Slightly substituted polymers have surface-active properties and can be valorized in various applications. Synytsya et al. (2004) prepared a number of N-alkylamides of highly methylated pectin used as bioavailable sorbents and drug delivery systems.

## Material and Methods

### Plant material

*The fruits of Diospyros peregrina* used in the study were collected at different stages (four stages viz., immature green, mature green, colour initiation and full ripe) of fruit ripening from the Forest Research Institute, Dehradun. All the chemicals used were of analytical grade.

### Pectin extraction and fractionation

The cell wall polysaccharides from fruit pericarp (6.174g) was grinded to a powder form and treated with PAW (Phenol:Acetic-acid:Water) in 2:1:1 ratio(w/v/v). Then it was washed with AIS (Acetone Insoluble Solid) within 5 min at 4˚C. After that, added 5 mg/ml of 50 mM CDTA and 50 mM Sodium acetate (pH 6.5) to it. This was then centrifuged for 6 hrs at room temperature by Magnetic-stirrer and filtered by GF/A filter paper. After filtration, through a GF/A filter paper, residue material was added to 5 mg/ml of 50 mM Sodium carbonate and 20 mM Sodium borohydride and kept for 20 hrs at 4˚C, called 1st-step. The filtrate is known as 1st Fraction. This 1st Fraction was precipitated with alcohol and then dried, the obtained dried yield known as Chelator soluble pectin fraction.

In the 2nd step, the remaining portion of 1st step was centrifuged by Magnetic-stirrer for 2 hrs at room temperature and filtered by GF/A filter paper. After filtration, through a GF/A filter paper, the residue material was added to 4 M KOH and 20 mM Sodium borohydride for 2 hrs at room temperature. After filtration, the left over filtrate, 2nd Fraction was precipitated with alcohol and dried, the obtained dried yield is known as Carbonate soluble pectin fraction. After treatment with 4 M KOH and 20 mM sodium borohydride, it was filtered by GF/A filter paper. After filtration, through a GF/A filter paper, the filtrate known as Alkali soluble pectin fraction, the remaining residual portion known as Cellulosic residue fraction.

### Determination of sugar composition

The uronicacid (UA) and neutral sugar (NS) content of the pectin extracts was determined using respectively, meta-hydroxybiphenyl (m-HBP) (Thibault, 1979;Vanden Hoogenet al., 1998) and resorcinol (Monsigny, Petit, & Roche,1998) on microtitration plates (nunc, Maxisorp, VWR international S.A.S., Fontenay-sous-bois, France); further analysed with a microplate spectrophotometer (Opsis MR; Dynextechnologies, VA, USA). l- Arabinose and D-galacturonic acid (Sigma–Aldrich Chimie S.a.r.l., Saint Quentin Fallavier, and France) were used as standards. The UA content was directly determined with the m-HBP test.The NS quantification was calculated after correction of the interference due to uronic acid for the resorcinol assay. All analyses were performed in triplicate.

## Results and Discussion

The AIS from fruits *Diospyros peregrina* was extracted sequentially and was fractioned into four fractions - Chelator soluble pectic fraction, carbonate soluble pectic fraction, alkali soluble fraction & cellulosic residue fraction. The % yield of the fraction of AIS from fruit pericarp in four stages of fruit ripening, Immature Green, Mature Green, Colour Initiation & Fully Ripe stages are given in Table. Generally, pectins are soluble in water representing no or little binding to the other cell wall components. It is believed that pectins bind together by calcium bridges and form complex compound with calcium ions. Therefore, pectin can be extracted by using different chelating agents such as; oxalates, hexametaphosphate, EDTA, CDTA, EGTA, etc. At normal condition (room temperature and at neutral pH), the action of chelating agent i.e. CDTA removes all the Ca-bridges from the pectins, rendering its solubilization.

Generally, chelator soluble pectins are found abundantly in fruit, moderately in leafy vegetables. Pectins extracted with CTDA had a wider molecular weight range with a peak molecular weight. So that CDTA or ammonium oxalate was preferred for pectin extraction. CDTA solubilized more pectin from ripe fruit and these observations are conclusive about the fact that higher dissolution of pectin-rich middle lamella occur during ripening. These fractions have pectin and represented by high galacturonic acid content along with relatively high galactose and arabinose residues. Chelator soluble polysaccharides are usually pectin. CDTA soluble fraction of Acetone Insoluble Solids (AIS) samples were prepared for different stages of fruit ripening. Chelator soluble pectic fraction in four stages was maximum 1.21 ± 0.09% in colour initiation stage and minimum 0.56 ± 0.04% in immature green stage, it 1.03 ± 0.12% and 0.85 ± 0.05% in mature green stage & fully ripe stage respectively. The chelator soluble pectin fraction yield showed sharp initial increase from immature green to mature green stage and it increased further from mature green to colour initiation step, this may be due to initial formation and then due to its formation and conversion of carbonate coluble pectin fraction to chelator soluble pectin fraction due to activity of pectinesterase enzyme, and further there was decrease from colour initiation stage to fully ripe stage and this decrease is attributed to pectin degradation. The results showed that CDTA-soluble pectins fraction are the major polysaccharides that undergo drastic degradation during ripening, already reported for other fruits. The chelator-soluble pectin was majorly affecting the fruit texture where textural loss and pectin content are related to each other. Chelator-soluble pectins exist as cross linked polymer which formed by pectic acids that bind calcium ions, which is responsible for tissue firmness. Ripening involves the breakdown of tightly bound insoluble protopectin to soluble polyuronides, which are loosely bound to the cell wall, appears to influence the changes in cell wall polysaccharides. The highly branched pectic polysaccharides in the primary cell wall are available for hydration and degradation, and as a result their solubilization leads to marked decrease in neutral sugar side chains as well as textural softening. The total chelator-soluble pectin in *Diospyros peregrina* fruits decreased during ripening with a regular increase in soluble galacturonide, along with progressive textural softening. The carbonate soluble pectic fraction yield in four stages of fruit ripening was maximum 9.25 ± 0.34% in mature green stage and was minimum 3.18 ± 0.07% in fully ripe stage, and it was 7.21 ± 0.26% and 8.64 ± 0.38% in immature green stage & colour initiation stage respectively. The carbonate soluble pectin fraction yield showed sharp initial increase from immature green to mature green stage and then it decreased from mature green to colour initiation step and to fully ripe stage, this may be due to its initial formation up to mature green stage and then degradation from mature green stage to fully ripe stage due to activity of pectin degrading enzyme. Presence of carbonate soluble pectic fraction as major pectin component as compared to chelator soluble pectin was observed.

The results showed that $Na_2Co_3$-soluble pectins fraction are second major polysaccharides that undergo degradation during ripening, already been reported for other fruits. The alkali soluble fraction in four stages of fruit ripening was maximum 39.32 ± 3.11% in fully ripe stage and minimum 33.71 ± 2.43% in immature green stage, and 35.54 ± 3.85% and 36.61 ± 2.77% in mature green stage and colour initiation stage respectively. The alkali soluble fraction % composition increased with the fruit ripening, this may be due to its formation in initial stages and also in later stages its increase in composition due to pectin degradation by pectin degrading enzyme activity. The cellulosic residue fraction in four stages of fruit ripening was maximum 56.98 ± 5.07% in fully ripe stage and minimum 49.85 ± 4.88% in immature green stage, and it was 51.26 ± 5.34% and 51.73 ± 4.82% in mature green stage & colour initiation stage. The cellulosic residue fraction % yield increased with the fruit ripening, this may be due to its formation in initial stages of fruit growth and in later stages its increase in composition due to pectin degradation by pectin degrading enzyme activity.

## Conclusion

The four distinct pectin fractions according to their relative solubility were extracted from the fruit pericarp of cell wall polysaccharides of *Diospyros peregrina*. The chemical characterization showed that the 1st step pectin fraction called Chelator soluble pectin fraction is easily soluble in Cyclohexadiamine tetra-acetate Sodium acetate. The 2nd step pectin fraction called Sodium carbonate soluble pectin fraction which is simply soluble in Carbonate-sodium borohydride as residual solid material. The 3rd step pectin fraction called Alkali soluble pectin fraction which is simply soluble in KOH; another pectin fraction known as Alkali insoluble pectin fraction which is insoluble in KOH. The 4th step fraction called Cellulosic residue fraction as residual solid material. The yield % of chelator soluble pectic fraction, carbonate soluble pectic fraction, alkali soluble fraction and cellulosic residue fraction in immature green, mature green, colour initiation and fully ripe stages of *Diospyros peregrina* is given in the Table.

**Table Isolation and yield % of chelator soluble pectic fraction, carbonate soluble pectic fraction, alkali soluble fraction and cellulosic residue fraction in AIS from fruits of *Diospyros peregrina* in different stages of fruit ripening**

| | Immature Green (1st stage) | Mature Green (2nd stage) | Colour Initiation (3rd =stage) | Fully Ripe (4th stage) |
|---|---|---|---|---|
| Chelator soluble pectic fraction | 0.56± 0.04% | 1.03 ± 0.12% | 1.21 ± 0.09% | 0.85 ± 0.05% |
| Carbonate soluble pectic fraction | 7.21 ± 0.26% | 9.25 ± 0.34% | 8.64 ± 0.38% | 3.18 ± 0.07% |
| Alkali soluble fraction | 33.71 ± 2.43% | 35.54 ± 3.85% | 36.61 ± 2.77% | 39.32 ± 3.11% |
| Cellulosic residue fraction | 49.85 ± 4.88% | 51.26 ± 5.34% | 51.73 ± 4.82% | 56.98 ± 5.07% |

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# Effect of Alcoholic Extract of *Stevia rebaudiana* on Fertility of Female Albino Rats

**S.P. Singh***
Reproductive Biology Cell, Department of Zoology
D.B.S. (P.G.) College, Dehradun-248001, Uttarakhand, India
*E-mail: drspsingh1949@gmail.com

**Abstract-** *Stevia rebaudiana* (Bert), family-compositae, is extensively used world over as natural sweetner but, scientists are worried about its safe use. First report on estrous cycle disturbing activity (ECDA) of this plant was published in 'Science'by Planas and Kuo. This has stimulated us to investigate the effect of alcoholic extract of this plant on fertility of female albino rats. The studies showed that the oral administration of different doses (50, 100 and 200 mg/kg/day for 30 days) of *Stevia rebaudiana* leaf powder as 50% alcoholic extract caused histopathological changes in reproductive organs and lengthening of diestrous phase of estrous cycle. The effects were dose dependent. It showed increasing degeneration in ovarian and uterine organs like developing, maturing (Graafian) follicles in ovary and endometrium and uterine glands respectively. Significant reduction of genital organ weight showed anti-estrogenic and anti-ovulatory effect. It also caused estrous cycle disturbing activity (E.C.D.A.). The study has revealed the anti-fertility effect of *stevia rebaudiana* in adult female rats.

**Keywords:** Fertility control, Contraception, Herbal Drugs, Reproductive Biology, Genital organs and *Stevia rebaudiana*.

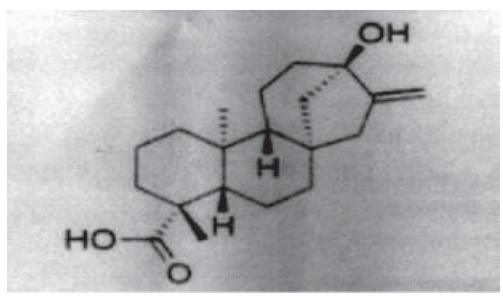
## Introduction

*Stevia rebaudiana* (Bertoni) family-compositae, is extensively used now a days world over as a natural non-sucrose sweetener. In Paraguay, it is used as hypoglycemic drug (Miquel, 1964). In the same country, Matto Gresso Indian tribal women drink daily Stevia decoction of leaves and stem as an oral contraceptive. Planas and Kuo (2011) reported contraceptive properties of stevia decoction including estrous cycle disturbing activity (E.C.D.A.) in female albino rats. Melis (1999) and Prasad et al. (1905) found reproductive disorders after feeding of aqueous extract of Stevia leaves at high doses in female and male albino rats. Misra et al. (2011) reported antidiabetic activity of leaves of Stevia on alloxan induced diabetic rats. Author has further studied anti-fertility effect of Stevia leaves as 50% alcoholic extract in female albino rats to confirm the ambiguous results as stated above.

## Phytochemistry

Chemists, Bridel and Lavielle (1931) isolated the glycosides, stevioside and rebaudioside that give the leaves their sweet taste. The extact structure of the aglycone steviol and its glycoside were published in 1955.

Stevia is widely grown for the sweet leaves which are the source of sweetener products known generically as Stevia and sold under various trade names. The chemical compounds that produce its sweetness are various steviol glycosides (mainly stevioside and rebaudioside) which have 250-300 times the sweetness of sugar. The leave can be eaten fresh or put in tea and food.




**Building Block-Steviol is the basic building block of stevia's sweet glycosides**

## Material and Methods

Adult and healthy Swiss female albino rats (weighing between 160 to 175 gms., colony bred in our department) were maintained under laboratory conditions providing them with standard diet (Hindustan Lever's Ltd.) and water ad libitum. They were divided into groups for each parameter and five rats were allotted for each dose. Control (5 rats) were also maintained for each parameter of study. Dried and powered leaves of *Stevia rebaudiana* were obtained from M/S Himachal Drugs Company, Dehradun. The 50% alcoholic extract of leave powder of Stevia was prepared using Soxhlet apparatus and 50% alcohol as solvent. The solvent was evaporated on water bath. Thus, the dried extract was used as drug. The doses 50, 100 and 200 mg/kg/of the extract were prepared by adding gum acacia powder as vehicle. Each dose was dissolved in distilled water in such a way that the dose was equivalent to 2ml of drug as solution and administered orally daily with the help of catheter fitted into a syringe-needle for 30 days. Control rats received vehicle only for the experimental period.

Swiss albino rats were maintained as per the protocol outlined in publications of the committee for the purpose of control and supervision of experiments on animals standard guidelines and approval obtained from Institutional Animal Ethical committee appointed by the Principal for laboratory animals.

### Body and Gental Organ Weight Changes

Both initial (start of experiment) and final (end of experiment) body weight of female rats were taken at the end of experiment (i.e. 30 days). After killing and autopsy (dissection), the genital organs i.e. ovary and uterus weight were noted. These organs were processed for histological study. The $P<0.05$ was used as variable for statistical analysis.

### Histopathological Studies

After administration of different doses (50, 100, 200 mg/kg/day) of stevia 50% alcoholic extract for 30 days to different groups (dose wise) of female rats, they were weighed and killed. Their ovaries and uteri were taken out, weighed and fixed in Bouin's fluid. These organs were processed for histological examination following standard method. Histopathological changes in treated rat's ovaries and uteri were compared with control ovaries and uteri of female rats. Photographs were also taken.

### Study of Effect of Estrous Cycle

During the above study of administration of three doses (50, 100, 200 mg/kg/day) for 30 days, the estrous cycle was studied daily following vaginal smear method of Kholkute et al, (1976) and compared with control rats. The vaginal smear of each rat was examined daily in the morning and estrous phases of cycle was recorded. The emphasis was given to diestrous (leucocyte cells) and estrous (cornified cells) phases of the cycle.

## Results and Discussion

### Body and Genital Organ Weight Changes

The results are presented in Table-1. The female rats of control group did not show any change i.e. reduction in the body weight. It was maintained throughout the experimental period. Similarly, no significant reduction in body weight was observbed at any dose level of Stevia alcoholic extract treatment for 30 days. However, the genital organ weight was reduced significantly ($P < -0.05$) after the treatment at 100 and 200 mg/kg doses for 30 days.

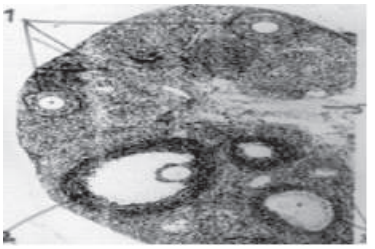
### Histopathological Changes

### Ovaries

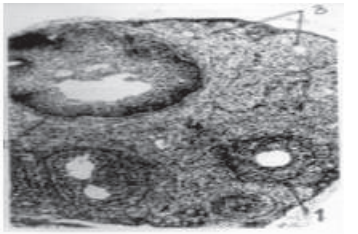
The structural organization of the ovaries of control female rats was presented normal features as evidenced by presence of all types of follicles, a few atretic follicles with normal vascularity in compact stroma. The germinal epithelium was intact (Figure-1). The dose 50 mg/kg/day was not very effective in causing untoward changes in ovary where as 100 and 200 mg/kg. doses for administration of 30 days caused deleterious effects on ovarian structural organisation. The large number of developing as well as mature follicles underwent atresia. The stroma was compact but vascularity was poor. The germinal epithelium was atrophied and devoid of primordial oocytes (Figure-2).

**Uteri**

The histological structure of uterus of control female rats did not show abnormality in organization. The endometrium was provided with large epithelial cells having basal and middle nuclei. The uterine glands were numerous, irregular and tortuous. The lumen appeared fully distended, and normal vascularity (Figure-3). The dose dependent effect was noted in the uterine histo-architecture. The higher doses i.e., 100 and 200 mg/kg/caused severe histopathological changes in uterine glands. They became less tortuous and non-secretory. The endometrial height and uterine lumen were reducted drastically. The myometrium was also effected. The stroma became compact, less vascular and non-secretary uterine gland. (Figure-4)

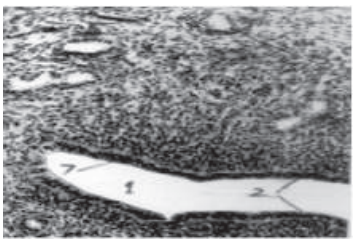


(Figure-1)
T.S. of ovary of control albino Rats with normal histological structure, all kind of follicles, stroma and vascularity X.60.



(Figure-2)
T.S. of ovary of treated albino rat with *S. rebaudiana* 100 and 200 mg/kg for 30 days. Note the arrest of follicle development and degeneration of other parts, follicular atretia X. 60.

Abbreviation –
(i) Developing follicle
(ii) Antric follicle
(iii) Atretic follicle
(iv) Stroma
(v) Vascularity



(Figure-3)
T.S. of Uterus of control albino rats with normal histological structure, normal endometrium, columnar epithelium, loose stroma, vascularity and uterine glands. X 100.

(Figure-4)
T.S. of Uterus of treated albino rats with *S. rebaudiana* 100 and 200 mg/kg for 30 days. Note histopathological changes as reduced endometrium, atrophied uterine glands and loss of vascularity. X 100.

Abbreviation –
(i) Uterine lumen
(ii) Endometrium
(iii) Uterine gland
(iv) Stroma
(v) Musculature

**Estrous Cycle Changes**

The cyclicity of control female rats group observed for 30 days was found normal with proper duration of each phase. The estrous cycle was perfectly alright (Table-2). In experimental groups, the administration of decoction of Stevia leaves at 50 mg/kg/day for 30 days caused lengthening of diestrous phase in 40% of rats with duration of 4.85

**Anti-fertility Effect by *Stevia rebaudiana***

Many plants with medicinal properties have been described to possess anti-fertility properties (Chaudhary, 1966 and Farnsworth et al. 1975). Search is still going on to assess the anti-fertility effect of Indian plants. *Stevia rebaudiana* leaves powder is considered as a safe sweetner in the place of commercial sugar. The decoction (Planas and Kuo, 1968) and aqueous extract (Prasad et al. 2005) of leaves of this plant were reported earlier to affect reproductive potential of female and male albino rats respectively. In the present study the leaves of 50% alcoholic extract of Stevia has shown its adverse effect on various reproductive functions of female albino rats. The effect was dose dependent.

The doses, 100 and 200 mg/kg for 30 days of administration caused significant reduction in genital organ weight ($P<0.05$), anti-implantation, estrous cycle disturbance and anti-ovulation which clearly demonstrate the estrogen-progesteron imbalance of secretion of gonadal hormone affecting hypothalamo-hypophysial axis (Jacob and Morris, 1969) Atrophic changes in ovary and uterus caused due to administration of Stevia leaves which disturbed the reproductive physiology appears to be directly affecting on these organs. The functional and structural changes in the uterus caused anti-implantation effect. The present study on histopathological changes in female genital organs are comparable to the studies made by Chakraborti et al. (1968) when female albino rats fed with green leaves of *Artobotrys odoratissimus* Linn. Follicular atresia and other degenerative changes in ovary which have similarity with present observation, were reported by Kholkute and Udupa (1974, 1976) and Kholkute et al. (1976) with the administration of *Hibiscus rosa sinensis* flower extract. Both ponderal (weight changes) and histopathological changes in the uteri were reported by Prakash (1979 a, b) respectively by administration of extract of *Embelia ribes.* Burm. seed in female albino rats. Singh and Singh (1992), also reported similar histopathological changes in ovary and uterus after administration of *Cassia fistula* flower extract in female albino rats within 30 days at different doses. The dose 50 mg/kg/days for 30 days of administration did not caused histopathological and other changes in reproductive organs of female albino rats but 100 and 200 mg/kg doses caused deleterious in the present study.

The results reported on the study of Stevia leaves in the present study, are similar with results reported by Dixit (1977) by administration of *Malva viscus conzatti* flower extract in female genital tract of Indian jerbil (*Meriones hurrianae* Jerdon). All such plants which have shown anti-fertility effect in female rats are suggestive to have phyto-estrogen and acts as anti-estrogen (also anti-progestational) in animals. It is concluded that *Stevia rebaudiana* (Bert.) have phyto-estrogen affecting the fertility of female animals.

## Acknowledgement

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**Table-1 Effect of Stevia leaves 50% alcoholic extract on body and genital organ weight of female albino rats administered for 30 days. The values are mean ± s.e.**

| Doses Mg/Mg | Body Weight (gms.) | | Genital organ weight (mgs) | |
|---|---|---|---|---|
| | Initial | Final | Ovary | Uterus |
| Control | 168.30 ± 3.20 | 173.30 ± 3.36 | 82.00 ± 1.15 | 105.25 ± 3.20 |
| 50 | 165.20 ± 3.20 | 172.15 ± 2.20 | 80.25 ± 2.30 | 100.00 ± 1.20 |
| 100 | 170.15 ± 2.20 | 175.20 ± 1.30 | 75.10 ± 3.15 * | 60.20 ± 2.17 * |
| 200 | 160.15 ± 4.10 | 161.30 ± 2.45 | 40.15 ± 1.20 * | 40.15 ± 1.20 * |

* $P<0.05$

**Table-2 Effect of Stevia leaves 50% alcoholic extract on estrous phases of estrous cycle of virgin female rats administered for 30 days. The values are mean ± s.e.**

| Doses Mg/kg | Number of rats used | Number of rats showing lengthening of diestrous phase | Percentage (%) of activity | Average duration of diestrous phase in days |
|---|---|---|---|---|
| Control | 5 | 0 | Nil | 0.45 ± 0.15 |
| 50 mg | 5 | 2 | 40 | 4.85 ± 0.75 |
| 100 mg | 5 | 4 | 80 | 8.80 ± 1.709 |
| 200 mg | 5 | 5 | 100 | 9.25 ±0.40 |

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# *In vitro* Antioxidant and Phytochemical Investigations of Ethanolic Extracts of *Viola serpens and Morus nigra*

**Anu Kumar[1], P. K. Chauhan[2], and I.P. Pandey[3*]**

[1]*Department of Biotechnology, HILS, Paonta Sahib, H.P., India*

[2]*School of Bioengineering and Food Technology, Shoolini University, Solan, H.P., India*

[3]*Prof. Emeritus Chemistry, Dehraun, UK., India*

**E-mail: ippande@gmail.com*

**Abstract-** This study investigates the influence of extraction system on the extractability of phytochemical compounds and antioxidant activity of ethanolic extracts of *Viola serpens* (Hindi name: Banafsha) and *Morus nigra* (Hindi name: Shehtoot). It can be concluded that the solvent used affects significantly the phytochemical content and the antioxidant activity of the extract and therefore, it is recommended to use more than one extraction system for better assessment of the antioxidant activity of natural products. Several of the investigated herbs contain substantial amounts of free radical scavengers and can serve as a potential source of natural antioxidants for medicinal and commercial uses. The phytochemical screening of ethanolic extract of *M. nigra* showed the presence of tannins and terpenoids where as alkaloids, saponins, flavonoids, amino acids, reducing sugars and glycosides were absent. Ethanolic extract of *V. serpens* showed the absence of alkaloids, saponins, tannins, terpenoids and glycosides, flavonoids amino acids and reducing sugars. The antioxidant screening of ethanolic extract of both plants showed the presence of enzymatic antioxidants such as catalase, peroxidase and ascorbate oxidase and non-enzymatic antioxidant such as ascorbic acid. The present study revealed that out of two working plants, *V. serpens* is more effective in its medicinal value.

**Keywords:** Antioxidants, Phytochemicals, Medicinal plants

## Introduction

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different parts of the country. In India, thousands of species are known to have medicinal values. The medicinal action of plants are unique to a particular plant species, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct[1]. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases**.** In search of novel sources of antioxidants, medicinal plants have been extensively studied for their antioxidant activity. From ancient times, herbs have been used in many areas including nutrition, medicine, flavoring, beverages, cosmetics, etc. The ingestion of fresh fruits, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases[2]. The higher intake of plant foods correlates with a lower risk of mortality from these diseases. Antioxidants are substances that are able to prevent or retard oxidation of lipids, proteins and DNA; and to protect the compounds or tissues from damage caused by oxygen or free radicals. Therefore, their health promoting effects reduce the risk of various diseases[3]. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases[4]. Many

studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables[5]. As many antioxidants are supplied from the diet, attention has been paid to the intake of the micronutrient antioxidants (vitamins A, C, and E, polyphenols and carotenoids) and to know how it may help to protect individuals from an oxidizing environment and/or inflammatory airway diseases. Approximately 60 % of the commercially available anti-tumoral and anti-infective agents are of natural origin[6] . The objective of this study was to investigate the scavenging capacities towards superoxide anion radicals and reducing power of the Berry's anthocyanin extract as a potential source of natural functional substances for use as dietary antioxidants. Many plant species have been utilized as traditional medicines, but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs[7].

## Material and Methods

### Collection of plant materials

The plant material used was dried leaves of medicinal plants which were *Viola serpens and Morus nigra* collected from the forest region of Paonta Sahib, H.P., India and were identified by Botanical Survey of India, Dehradun, India.

### Extraction of plant material

The plant material taken for the study was stored under refrigerated condition till use. The samples were prepared by extraction of plant material with ethanol solvent by Soxhlet apparatus. By evaporating on water bath, a crude extract was obtained of medicinal plants.

### Storage

Plant extracts were stored at the temperature of 40ºC till the use for investigation.

### Phytochemical Investigations

Alkaloid and Saponin and Flavonoids were investigated according to the procedure given by Harbone, J.B.[8]

Tannins were investigated according to the procedure given by Trease, G.E. et. al[9].

Glycoside and Terpenoids were investigated according to the procedure given by Siddiqui, A.A.[10]

Reducing sugar and Amino acids were investigated according to the procedure given by Harbone J.B.[8]

### Determination of antioxidant activity

Assay of Catalase activity: Catalase activity was assayed by the method given by Sinha, A.K.[11]

Assay of Peroxidase activity: The assay was carried out by the method given by Addy, S.K. et. al[12]

Assay of ascorbate oxidase activity: Assay of ascorbate oxidase activity was carried out according to the procedure of Vines, H.M. et. al[13]

Quantification of vitamins: The determination of ascorbic acid was carried out by the procedure given by Sadasivam, S. et. al[14]

## Results and Discussion

### Phytochemical Screening of Plant Materials

The phytochemical screening of ethanolic extract of *M. nigra* showed the presence of tannins and terpenoids where as alkaloids, saponins, flavonoids, amino acids, reducing sugars and glycosides were absent. Ethanolic extract of *V. serpens* showed the absence *o*f alkaloids, saponins, tannins, terpenoids and glycosides, flavonoids amino acids and reducing sugars (Table-1) Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of blood lipids. Thus, these plants have been used to lower the blood lipid content.

**Table-1 Phytochemical constituents of *Viola serpens* and *Morus nigra***

| Phytochemicals | *M. nigra* | *V. serpens* |
|---|---|---|
| Alkaloids | -- | -- |
| Saponins | -- | -- |
| Tannins | + | -- |
| Amino acids | -- | + |
| Terpenoids | + | -- |
| Reducing sugars | -- | + |
| Glycosides | -- | -- |
| Flavonoids | -- | + |

**Antioxidant Activity of Ethanolic Extracts of *Morus nigra and Viola serpens***

The levels of antioxidant enzymes assessed in both the plants. The ethanolic extracts were collectively represented in Table-2. Among the ethanolic extracts of both plants the highest activity of Catalase was observed in the ethanol extract of *Viola serpens* (0.40 units/mg protein) and lowest in ethanolic extract of *Morus nigra* (0.14 units/mg proteins).

**Table-2 Enzymatic Antioxidant Analysis in ethanolic extracts of *Morus nigra and Viola serpens***

| Samples | Catalase μ/moles of H2O2 decomposed /min/g extract | Peroxidase 1U/L | Ascorbate oxidase μ mole /ml |
|---|---|---|---|
| Ethanolic extracts of *Morus nigra* | 0.14 | $4.46 \times 10^3$ | 1.855 |
| Ethanolic extracts of *Viola serpens* | 0.40 | $11.9\times 10^3$ | 0.153 |
| | 1 unit = μ/moles of H202 decomposed /min/g extract | 1 unit = μmoles pyrogallol oxidized/ min | 1 unit = 0.01 O.D change /min |

In plants, antioxidant enzymes, namely catalase[15] and peroxidase have been shown to increase when subjected to stress conditions. The Peroxidase activity was observed to be low in ethanolic extract of *Morus nigra* (4.46 × 103 units/mg protein), while the activity increased in ethanolic extract of *Viola serpens* (11.9× 103). The ascorbate oxidase activity was highest in ethanolic extract of *Morus nigra* (1.855 units/mg protein) and lowest in the ethanolic extract of *Viola serpens* (0.153 units/mg protein). The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity[16]. The reducing ability of a compound generally depends on the presence of reductant which possesses the antioxidative potential by breaking the free radical chain by donating a hydrogen atom. Ethanolic extract *A. lamarckii* and its sub-fractions exhibited a good reducing power. Leaves of this plant are useful for curing diabetes. Decoction of bark has been used as an emetic in India. Methanol extract of *Alangium salviifolium* flowers has shown antibacterial activity against both gram-positive and gram-negative bacteria. Methanolic extract of root of *A. salviifolium* have shown analgesic and anti-inflammatory activities in albino mice[17].

**Non–Enzymatic Antioxidant Activity (Ascorbic acid)**

The concentration of non-enzymatic antioxidant (Ascorbic acid) in ethanolic extracts of both plants was also assessed and the results are represented in **Table-3**.

**Table-3 Non–Enzymatic Antioxidant Activity (Ascorbic acid)**

| Samples | Vitamin C (mg/g) |
|---|---|
| Ethanolic extract of *Morus nigra* | 0.173 |
| Ethanolic extract of *Viola serpens* | 0.051 |

Vitamin C content was high in the ethanolic extract of *Morus nigra* (0.173 mg/ g tissue), whereas in ethanolic extract of *Viola serpens*, it was (0.051 mg/ g tissue). Ascorbate has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells and shown to function as a reductant for many free radicals [18]. Oxidative damage to cellular components such as lipids and cell membranes by free radicals and other reactive oxygen species is believed to be associated with the development of a range of degenerative diseases, including heart diseases, cancer, inflammation, arthritis, immune system decline, brain dysfunction. Blackberries are a good source of anthocyanins in which the anthocyanin contents were reported to be 67.4–230 mg/100 g fresh weight[19]. Furthermore, the anthocyanin pigment in blackberry has also exhibited a strong scavenging activity towards nitrite and thereby prevents the formation of nitrosamine and reduces the carcinogenesis induced by nitrosamines. Therefore, the anthocyanin pigment in blackberry is a natural, edible colorant with excellent antioxidant properties and health benefits and seems applicable in both healthy food and medicine. Both the plants may play an important role in the prevention of human diseases related to oxidative damage.

## Conclusion

The results of the present study revealed that the ethanolic extracts of both the plants, i.e. *Morus nigra* and *Viola serpens* have antioxidant properties since these contains enzymatic and non -enzymatic antioxidants. These can be very effective against microbes causing various diseases. *In vitro* assessment of the antioxidant activity of ethanolic fractions of *M.nigra* and *V.serpens* to scavenge 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and highly reactive hydroxyl radicals showed that the semi - pure compounds present in the fractions are useful potential source of antioxidants and can be used in the therapy of diseases like cancer, coronary heart disease, ageing and any other disease related to oxidative stress. These fractions being non-toxic showed significant antioxidant activity at scavenging free radicals. They also significantly scavenge hydroxyl radical which is known to cause cellular damage.

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# Degradative Impact of Fungal Infestation on Some Physicochemical and Biochemical Attributes of *Pongamia pinnata* (L.) Pirre Seeds

**Tarannum[1], Nishat Anjum[2*] and Y.C. Tripathi[2]**

[1]Department of Science, Kamran MANUU Model School, Maulana Azad National Urdu University, Darbhanga-846001, Bihar, India

[2]Chemistry Division, Forest Research Institute, PO. New Forest, Dehradun-248006, Uttarakhand, India

*E-mail: nishatanjum55@gmail.com

**Abstract-** The study was aimed to investigate the impact of long-term storage and resultant fungal invasion on physicochemical and biochemical characteristic of *Pongamia pinnata* seed oil. Seeds were stored in gunny bags for 180 days at room temperature and change in physicochemical and biochemical attributes of seeds and seed oil subsequent to their storage were investigated at an interval of 60 days for 180 days. Altogether six dominant fungi were isolated and identified as *Alternaria alternata, Aspergillus candidus, A. flavus, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus nigricans* based on growth characteristic, mycelial morphology, spore morphology and other important characters. Results of the study showed the initial moisture content of the seeds increased and subsequently decreased whereas ash value and crude fibre increased till the end of storage period. Fatty oil, crude protein and carbohydrate showed a consistent decreasing trend throughout the storage duration. The acid value of the seed oil constantly increased to a higher value all through at the seed storage period. Further gradual increase in soponification value and decrease in iodine number of the fatty oil was observed all over the storage duration. The results are in agreement with the previous studies on similar aspects with various other oilseeds.

***Keywords*:** *Pongamia pinnata,* Seeds, Biodeterioration, Physicochemical values, Biochemical traits.

## Introduction

Tree borne oil seeds like other plant products, harbour a variety of microorganisms which get associated either in the field or in the post harvest storage (Singh *et al.*, 1999). Under certain conditions the fungi start growing and consuming seeds for growth and reproduction and their activity causes undesirable change of varied nature in the seeds including quality of nutrients, loss of constituents nutrients, poisoning of the products by mycotoxins, loss of germinability. Fungi growing on stored seeds, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces increased moisture content, free fatty acid content and enhancing other biochemical changes (Bhattacharya and Raha, 2002; Kakde and Chavan, 2011). The species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Alternaria* are found commonly occurring as post harvest moulds in storage condition. Most of the species of *Aspergillu*s are dominant and play vital role in the seed biodeterioration (Harman, 1983). The problem of rancidity is common in most oil seeds under poor storage conditions. Fungal infestation of stored oil seeds may cause rancidity resulting into loss of quality and poor market value (St. Angelo and Ory, 1983). Rancidity is an important quality factor in seed oil for various industrial applications. Several study reports had reported reduction in oil, protein and carbohydrate content, as well as increased free fatty acid and peroxide values in fungal-infected oil seeds (Bhattacharya and Raha, 2002; Negedu *et al.*, 2010; Begum *et al.*, 2013; Saxena *et al.*, 2015).

*Pongamia pinnata* (L.) Pierre is a small evergreen glabrous tree belonging to *Fabaceae* popularly known as Karanja in Hindi and Indian Beech in English. This legume tree is native to Bangladesh,

India, Pakistan, Myanmar, Nepal, Thailand and also distributed throughout Australia, China, Egypt, Fiji, Indonesia, Japan, Malaysia, Mauritius, New Zealand, Pakistan, Papua New Guinea, Philippines, Samoa, Seychelles, Solomon Islands, Sri Lanka, Sudan, Tonga and USA (Kirtikar and Basu, 1994). The plant has long been used as a source of animal fodder, fuel, manure, fish poison, timber and traditional medicine. Fatty oil obtained from seeds of *P. pinnata* is used for illumination, as a kerosene substitute, water paint binder, pesticide. *P. pinnata* seed oil is known for its multipurpose benefits and as a potential source of biodiesel (Azam *et al*., 2005; Karmee and Chadha, 2005; Naik *et al.*, 2008). The seeds of *P. pinnata* contain 30 to 40% oil known as Pongam oil or Pongamol or Hongay oil (Natanam *et al.*, 1989; Nagaraj and Mukta, 2004) with high percentage of polyunsaturated fatty acids (Sarma *et al.*, 2005) which can be converted to biodiesel by transesterification method (Meher *et al.*, 2006). On account of high fatty oil content, it is believed that *P. pinnata* seed oil would fulfil the demands of biofuel in India (Punia *et al.,* 2006). It has been recognized as biodiesel as several parameters of diesel and *P. pinnata* seed oil are comparable (Gerphen *et al.*, 2004; Shaine *et al.*, 2004). Seeds of *P. pinnata* are subjected to attack by different fungal species such as *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. and cause seed deterioration leading to poor germination, survival and oil yield (Pandey and Prasad, 1993; Kumar *et al*., 2007, Arya *et al.*, 2015). Therefore, the study was designed to evaluate the deterioration in terms of changes in certain physicochemical and biochemical parameters of *P. pinnata* seeds under the influence of fungal infestation during storage.

## Material and Methods

### Collection and storage of seeds

Pods of *P. pinnata* were collected from suburbs of Dharbhanga, Bihar, India and identified by the Systematic Botany expert. Collected pods were thoroughly washed under running tap water in order to remove dust from their surface and dried. Seeds were separated from the pods by decortication. They were then stored in gunny bags for 180 days at room temperature.

### Isolation and Identification of Fungi

The isolation of fungi from the stored *P. pinnata* seeds was carried out by blotter test method (ISTA, 2003). A pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized petridishes of 9 cm diameter. Stored seeds of *Pongamia pinnata* were placed at equidistance on moist blotters in autoclaved separate petridishes. All petridishes were incubated at room temperature ($28\pm2^{0}$C) for 6 days. Fungi were isolated and indentified standard using standard protocol (Barnett and Hunter, 2000; Mukadam *et al.*, 2006).

### Evaluation of Physicochemical and Biochemical Traits

The change in physicochemical and biochemical attributes of *P. pinnata* seeds and seed oil subsequent to their storage were investigated at an interval of 60 days for 180 days. These investigations were carried out in freshly harvested seeds samples as well. The moisture, total ash, fatty oil, crude fibre, crude protein and carbohydrate contents were determined as per the standard method (AOAC, 1995). The oil was extracted with hexane in a Soxhlet apparatus. The solvent was then removed using a rotary vacuum evaporator at reduced pressure and the percentage of oil was determined. Crude protein was determined by estimating nitrogen content by microkjeldahl technique. The amount of N content was multiplied by 6.25 factors which gave crude protein content of the samples. The acid value, saponification value and iodine value of the seed oil were also evaluated using standard methods of AOAC (1995). All determinations were replicated thrice and expressed as mean± standard error of M (SEM).

## Results and Discussion

### Identification of Fungi on Stored Seed

Altogether six dominant fungi were isolated and

identified as *Alternaria alternata, Aspergillus candidus*, *A. flavus*, *Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus nigricans* based on growth characteristic, mycelial morphology, spore morphology and other important characters. It was observed that there was a gradual increase in the storage fungi with progression of storage period. Seeds are highly susceptible to fungal invasion, proliferation and elaboration of mycotoxins during storage which is one of the major factors for the low productivity of oil.

**Changes in Physicochemical and Biochemical Traits**

The effect of fungal infestation on the parameters studied of *P. pinnata* seeds including moisture, total ash, fatty oil, crude fiber, crude protein, carbohydrate, acid value, saponification value, and iodine number after 0, 60, 120, 180 days of storage were studied and the result are presented in Table-1.

**Table-1 Physicochemical and biochemical values of *P. pinnata* seeds after different storage period**

| Physicochemical & Biochemical Traits | Values (Mean? SE) after different storage periods (Days) | | | |
|---|---|---|---|---|
| | 0 | 60 | 120 | 180 |
| Moisture (%) | 9.43 ? 1.01 | 10.85 ? 1.82 | 10.16 ? 0.63 | 9.88 ? 1.23 |
| Ash value (%) | 2.35 ? 0.87 | 3.53 ? 1.12 | 5.14 ? 1.05 | 5.96 ? 1.06 |
| Fatty oil (%) | 33.65 ? 2.17 | 28.47 ? 0.93 | 25.16 ? 1.16 | 19.68 ? 1.18 |
| Crude fibre (%) | 7.50 ? 1.12 | 11.33 ? 1.03 | 17.38 ? 1.01 | 21.25 ? 0.96 |
| Crude protein (%) | 18.22 ? 0.83 | 16.84 ? 1.15 | 15.26 ? 1.33 | 12.55 ? 2.06 |
| Carbohydrate (%) | 10.58 ? 1.05 | 9.95 ? 0.83 | 7.66 ? 1.27 | 5.23 ? 1.23 |
| Acid value (mg KOH/g) | 3.66 ? 1.12 | 4.23 ? 1.06 | 5.87 ? 1.05 | 7.25 ? 1.12 |
| Saponification value (mg KOH/g) | 185.32 ? 0.65 | 187.28 ? 1.12 | 188.86? 1.05 | 190.54? 1.15 |
| Iodine number (g/100 g) | 92.58 ? 1.15 | 90.35 ? 1.17 | 87.48 ? 1.05 | 84.25? 1.23 |

Moisture content of seed plays an important role in influencing the fungal colonization during the storage. Variations in the chemical composition of the seeds are known to be influenced by the seed moisture content. In the present study, the initial moisture content of the seeds increased and subsequently decreased till the end of storage period (Table-1). A similar trend was observed in previous studies with groundnut and soybean reporting initial increase in moisture content of fungi-infected stored seed, followed by a gradual decrease during prolonged storage (Bhattacharya and Raha, 2004). It is due to the fact that seeds under storage may absorb or lose moisture till the vapour pressure of seed moisture and atmospheric moisture reach equilibrium (Shelar *et al.*, 2008).

The ash value of seeds gradually increased till the end of storage period as evident from the date in Table-1. The increase in ash content could be attributed to the presence of certain mineral elements (such as K and P) in the mycelium of the test fungus growing on the stored seeds. The finding is in agreement with the similar report on seeds of *Irvingia gabonensis* (Ataga and Ota-Ibe, 2006).

Considerable decrease in fatty oil, content in the stored seeds is recorded throughout the different

period of storage. The reduction in fatty oil content of fatty oil rich *P. pinnata* seeds is generally attributed to utilization of total fat by the colonizing fungi over the storage period. The results are in agreement with the previous research findings on similar aspect (Tripathi *et al.*, 1996a; Bhattacharya and Raha, 2004; Bankole *et al.,* 2005).

A sharp increase in crude fibre content throughout the storage period of seeds was recorded which is in conformity with the findings on crude fiber content of fungal infected peanuts in storage (Horn, 2005). The increasing trend in the fibre content may be due to the ability of the fungus to produce necessary enzymes required for the hydrolysis of the structural carbohydrates such as cellulose and hemicellulose into their component monomers. Some of the polysaccharides might have been retained as crude fiber leading to the higher value in the crude fiber content as shown in Table-1.

Continual decline in crude fibre and carbohydrate contents as compared to fresh was noted with progression of seed storage period (Table-1). Decrease in protein content may be due to breaking down of protein into amino acids under the influence the fungal action during storage (Sinha and Prasad, 1977). Similar observations were found in stored vegetable seeds (Sethumadhava *et al.*, 2014) and stored red and black gram seeds. Further decrease in carbohydrate may be due to fungi utilized sugar as a substrate for its growth.

The acid value of the fatty oil extracted from stored seeds constantly increased to a higher value all through at the seed storage period (Table-1). The increase in acid value could be due to the conversion of the oil into fatty acids as observed (St.Angelo and Ory , 1983). It could also be due to production of the free fatty acids at a rate faster than that utilized by the associated fungi growing on the seeds resulting in an increased free fatty acid level. A similar observation was recorded with melon seeds (Bankole *et al.,* 2005) and stored palm fruits (Oso, 1979). It is reported that some fungi utilized fatty acids as sole carbon sources for energy and growth (Negedu *et al.*, 2010). Such fungi produced lipases which hydrolyzed the ester linkages of the oil to free the linked fatty acids thereby giving rise to an increased level of the acid value.

Gradual increase in soponification value and decrease in iodine number of the fatty oil extracted from seeds was observed all over the storage duration. These changes may be due to the formation of number of short chain fatty acid glycerides during the lypolysis of oil by the enzyme lipase. The relation between Iodine number and soponification values is an important criterion for the assessment of quality of oils. Iodine number indicates the quantity of unsaturated acids present in the oil. However, reports are contradictory as far as impact of fungal infestation on iodine number of fatty oils is concerned. Lalithakumari (1971) observed a low Iodine number in oil extracted from infested groundnut seeds; on the contrary, Ward and Diener (1961) reported an increase in iodine number in peanut owing to fungal invasion.

Several reports reveal that storage fungi cause changes in physicochemical and biochemical characteristic of seeds. Oil yielding crop plants are very important for economic growth of the energy and agricultural sectors. The oil seeds containing polyunsaturated fatty acids are important source of biodiesel (Sharmin *et al.*, 2006). These organic seed oils are better than diesel fuels in terms of physico-chemical properties and biodegradability (Scott *et al.*, 2008). The quality of fatty oils is dictated by several physical and biochemical parameters that are dependent on the source of oil, geographic, climatic, and agronomic variables of growth as well as processing and storage conditions (Tripathi *et al.*, 1996b, 1997, 1998; Rathore *et al.*, 1998; Shahidi, 2005). Deterioration of oilseeds due to improper storage conditions cause loss of yield and quality, thereby poor market value. In many parts of the world, production of Karanja seed oil for use as biodiesel is gaining popularity (Nagalakshmi *et al.,* 2011)

## Conclusion

Seeds of *P. pinnata* have gained a great commercial relevance owing to their high oil content and suitability as an alternate source of fuel and energy. In developing countries such as India, there is an increasing demand for fuel to meet everyday transportation needs. Shrinking reserves of fossil fuel and resultant increasing price of petroleum products may have serious implications for the automobile industry in the future. Therefore, search for an alternative to the fossil fuel from renewable natural resources has become imperative (Srivastava and Prasad, 2000). Increased use of renewable and eco-friendly biofuels may also minimize the import of crude oil. However, as per standards deteriorated oilseeds cannot be acceptably used for industrial purposes. *P. pinnata* is one of the promising candidates for bio-diesel production which are renewable, safe, non-polluting and eco-friendly. It is, therefore to attain increased production of good quality oil; a special attention is warranted on information regarding the biodeterioration of the oilseed to met specifications for purity and quality.

**Conflict of Interest:** None declared

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# About Flowers

*Syzygium jambolana* (Jamun)

*Aegle marmelos* (Bel)

*Balsamodendron mukul* (Guggul)

*Ocimum sanctum* (Tulsi)

***Syzygium jambolana* (Jamun)**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Order | : | Myrtales |
| Family | : | Myrtaceae |
| Genus | : | Syzygium |
| Species | : | jambolana |

**Jamun (*Syzygium jambolana*)** is a large evergreen tropical tree native to India. It is distributed throughout India up to an altitude of 1800 meters. The habitat of Jamun tree starts from Myanmar and extended to Afghanistan. This tree is also found in other countries viz., Thailand, Philippines and Madagascar. All parts of this tree are used in traditional medicine to treat various diseases. The bark of Jamun tree is astringent, sweet, refrigerant, carminative, diuretic, digestive, anti helminthic, febrifuge, constipating, stomachic and antibacterial. It is also a good blood purifier.

The bark of Jamun tree contains pentacyclic triterpenoid betulinic acid. Betulinic acid is a naturally occurring tri-terpenoid which has demonstrated selective cytotoxicity against a number of specific tumor and active against a variety of infectious agent such as HIV, malaria, etc. It also shows immunomodulatory and anti-inflammatory activities. All parts of this tree contains sterol-sitosterol which has same chemical structure with cholesterol. The bark is useful in lowering blood cholesterol and blood sugar. The presence of gallo- and ellagi-tannins in bark is responsible for its astringent property. The seeds (Jamun Guthali) of Jamun tree contains alkaloid, jambosine and glycoside jambolin or antimellin responsible for stopping diastatic conversion of starch into sugar. Seeds are also rich in flavonoids and phenolics which are antioxidant and scavenge free radicals from body. Jamun seeds are sweet, astringent to the bowels and good for treating diabetes.

***Aegle marmelos* (Bel)**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Order | : | Sapindales |
| Family | : | Rutaceae |
| Genus | : | Aegle |
| Species | : | marmelos |

***Aegle marmelos*** is a spinous and deciduous aromatic tree, straight, strong and axillary. It grows up to 18 meters tall and bears long thorns.As a medicine, it is used in diabetes, dyspepsia, chronic diarrhea, heart diseases, dysentery. Leaf: against peptic ulcers and respiratory disorders. Fruits: arrest secretion or blooding, cure diahorrea and dysentry. Root: ear problems.

***Aegle marmelos*** extract was found to significantly reduce serum glucose in laboratory animals. Laboratory rats were diabetes induced by administration of alloxan which is a toxic glucose analogue that destroys insulin producing cells by damaging islet cells of the pancreas by liberating oxygen radicals. *Aegle marmelos* leaf alcohol extract was used and found to have significantly reduced blood sugar in alloxan diabetic rats. Results could be seen after 6 days of continuous administration. On the 12th day, sugar levels were found to be reduced by 54%. Oxidative stress was found to be significantly lowered by the extract. This was evidenced by a significant decrease in lipid peroxidation, conjugated diene and hydroperoxide levels in serum as well as a reduced liver damage were found.

***Balsamodendron mukul* (Guggul)**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Order | : | Sapindales |
| Family | : | Burseraceae |
| Genus | : | Balsamodendron |
| Species | : | mukul |

***Balsamodendron Mukul*** is used for the treatment, control, prevention and improvement of the diseases, conditions and symptoms:Osteoarthritic pain, Nervous diseases, Leprosy, Muscle spasms, Urinary tract disorders. It decreases elevated serum levels of cholesterol,free fatty acids and triglycerides. **Guggul** has been used in the traditional Ayurvedic medical system for centuries and has been studied extensively in India. Commercial products are promoted for use in hyperlipidemia; however, clinical studies do not substantiate this claim. Anti-inflammatory and cardiovascular effects are being evaluated, as well as its use in cancer, obesity, and diabetes.

***Ocimum sanctum* (Tulsi)**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Order | : | Lamiales |
| Family | : | Lamiaceae |
| Genus | : | Ocimum |
| Species | : | sanctum |

***Ocimum sanctum*** commonly known as **holy basil**, ***tulasi*** (sometimes spelled ***thulasi***) or ***tulsi***, is an aromatic plant in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

*Tulasi* (Sanskrit:-Surasa) has been used for thousands of years in Ayurveda for its diverse healing properties. It is mentioned in the Charaka Samhita, an ancient Ayurvedic text. *Tulsi* is considered to be an adaptive balancing different processes in the body, and helpful for adapting to stress. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity. **O. sanctum** is a medicinal plant distributed mainly in the tropical and subtropical regions including India. It is widely used for its hypoglycaemic and antidyslipaedimic effects in diabetes. However, these effects were displayed only in short term studies. It stimulates endogenous insulin secretion by altering cell membrane.

# Forth Coming Events

1. Ieeeforum-International conference on medical biological and pharmaceutical sciences (ICMBPS)
   16 July 2017
   Mumbai, Maharashtra, India
   Website http://ieeeconference.com/Conference2017/7/Mumbai/ICMBPS1/

2. 10th Global Summit on Toxicology and Applied Pharmacology
   July 20-22, 2017
   Chicago, Illinois, USA
   http://toxicology.global-summit.com

3. The IRES - 114th International Conference on Food Microbiology and Food Safety(ICFMFS)
   25th July 2017
   Venue: Pune, Maharashtra, India
   Website http://theires.org/Conference2017/India/2/ICFMFS/

4. IASTEM-23 International Conference on Medical, Biological and Pharmaceutical Science (ICMBPS)
   25th to 26th August 2017
   Pune, Maharastra, India
   http://iastem.org/Conference2017/India/1/ICMBPS

5. 5th International Pharmacy Conference on Innovations in Exertions of Pharmacy
   August 31-September 01, 2017
   Philadelphia, USA
   http://pharmacy.pharmaceuticalconferences.com/

6. 9th World Congress on Pharmacology
   September 04-06, 2017
   Paris, France
   http://pharmacology.pharmaceuticalconferences.com/

7. New and Old Phytochemicals: Their Role in Ecology, Veterinary, and Welfare
   September 17 - 20, 2017
   Francavilla al Mare, Chieti, Italy
   http://psecongress2017uda.wixsite.com/main

8. The IIER-277th International Conference on Natural Science and Environment (ICNSE)
   25th to 26th September 2017
   Pune, India
   http://theiier.org/Conference2017/India/2ICNSE/

9. 3rd World Congress on Medicinal Plants and Natural Products Research on: Medicinal Plants as a Curative Medicine
   October 02-03, 2017
   Kuala Lumpur, Malaysia
   http://medicinalplants.pharmaceuticalconferences.com/

10. 2nd International Conference on Pharmaceutical Chemistry
October 02-04, 2017
Barcelona, Spain
Theme: Recent Trends and Advancements in the fields of Pharma and Chemistry
http://pharmaceuticalchemistry.conferenceseries.com/

11. 4th International Conference on Past and Present Research Systems of Green Chemistry
October 16-18, 2017
Atlanta, Georgia, USA
http://greenchemistry.conferenceseries.com/

12. 3rd International Conference on Natural Products Utilization: from Plants to Pharmacy Shelf (ICNPU-2017)
October 18 - 21, 2017
Bansko, Bulgaria
http://www.icnpu.com/2017

13. 2nd International Conference on Applied Chemistry
October 30-November 01, 2017
Chicago, Illinois, USA
http://appliedchemistry.conferenceseries.com/

14. 2nd International Conference on Applied Chemistry" (ICAC-2017) on Applied Chemistry"
November 25-28, 2017.
Hurghada - Egypt, from
http://isac-chem.sohag-univ.edu.eg/index.html

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Vigyan Dham, Jhajra, Dehradun - 248 007
PH. +91-135-2102769/70; Email: dg@ucost.in


## 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 Photochemistry and Ayurvedic Height for their endeavor in Herbal research.

**Dr. Rajendra Dobhal**
Director General

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