Phytochemical, Antioxidant and *In Vitro* Antibacterial Activity of Aqueous and Acetone Fruit Extracts of *Kigelia Africana*

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Abstract- Phytochemicals present in plants probably explain the various uses of plants for traditional medicine. In this study *Kigelia Africana* fruit was selected for assessing the level of various Phytochemicals, enzymatic and non- enzymatic antioxidants and antimicrobial activity. Results showed the presence of Alkaloids, Glycosides, Terpenoids & Flavonoids and Tannins in both the extracts whereas reducing sugars and saponins were absent in acetone fruit extract. The levels of different enzymatic and non- enzymatic antioxidants were also assessed in different extracts of *Kigelia africana* for catalase, peroxidase, ascorbate oxidase and vitamin C activities, which were found to be maximum in aqueous fruit extract. Antimicrobial activity of these extracts was then studied using agar well plate method against the bacteria strains isolated from the Urine samples of stone patients. Antimicrobial activity of aqueous extract was also greater than that of the acetone extract.

Keywords: *Kigelia africana*; phytochemicals; antioxidants; antimicrobial activity.

Introduction

Human use of plants as medicines agent pre-dates recorded history. Ethnomedicinal plant-use data in many forms has been utilized in the development of formularies and pharmacopoeias, providing major focus in global healthcare, as well as contributing substantially to the drug development process[1]. Secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds. The presence of these secondary metabolites in plants probably explains the various medicinal and antioxidant activities of these plants[2]. Antioxidants help to prevent the free radical damage that is associated with cancer and heart disease. *Kigelia africana* is a plant used for this study. It is found mostly in riverine areas where distribution is restricted to the wetter areas. Antibiotics are sometimes associated with adverse effects on hosts which include hypersensitivity, depletion of beneficial gut, mucosal microorganisms, immune suppression and allergic reactions. Bacteria have the genetic ability to transmit and acquire resistance to drugs[3]. Essential oils and extracts of certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods[4]. The investigation of certain indigenous plants for their antimicrobial properties is very useful[5] and there is increasing interest in plants as source of agent to fight microbial diseases and treatment of several infections[6]. This study mainly concerned with the phytochemical antioxidant and antibacterial activity of the aqueous and acetone fruit extracts of *Kigelia africana* using clinical isolates of bacteria from urine samples of stone patients.

Material and Methods

Collection of plant materials

The plant material used was the dried fruit of *Kigelia Africana* collected from FRI, Dehradun and were identified by Botanical Survey of India, Dehradun.

Extraction of plant material

The plant material taken for the study was stored under refrigerated condition till use. The extracts were prepared by using Sonicator and soxlet apparatus and evaporated on water bath then crude extracts were obtained and stored at the temperature of 40°C till use for investigation.

Phytochemical investigations

Tests for alkaloids and flavonoids

Tests for Alkaloids and Flavonoids were performed by the method of Harborne J B *et al.*[7].

Test for saponins

Foam test: 1ml of aqueous extract was diluted sepa-

rately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Test for tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic, tannins and green black for catecholic tannins.

Test for glycoside

Glycoside test was performed by the method of Siddiqui A A *et al.*[8].

Test for terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoids and green bluish color for steroids.

Test for reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitates.

Antioxidant activity

Catalase activity was assayed by the method of Sinha *et al.*,[9]. Peroxidase activity was carried out by the method of Addy and Goodman *et al.*[10]. Ascorbate Oxidase activity was carried out by the method of Vines and Oberbacher *et al.*,[11]. The ascorbic acid activity was carried out by the method of Sadasivam, S *et al.*,[12].

Determination of antibacterial activity

The antimicrobial activity of *Kigelia africana* fruit extract was carried out by using *in vitro* agar well diffusion method. Muller Hinton agar was sterilized by using autoclave, then it was poured in petri plates and allowed to get solidify. The wells of desired diameter (8 mm) were made with the help of borer. Bacterial suspension of each strain is applied and grown overnight. The fruit extract was poured in each particular labeled well with the help of sterile micropipettes by maintaining the asceptic environment. These petri plates were then kept for incubation at 37°C for 24hrs. After the completion of incubation period, the zones of inhibition were measured and recorded. The antimicrobial activity of *Kigelia africana* fruit extract was performed against bacterial strains i.e. *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris.*

Results and Discussion

Phytochemical investigation of *Kigelia Africana*

Qualitative analysis showed the presence or absence of phytochemical constituents and the results are summarized in **Table - 1.** Results showed the presence of Alkaloids, Glycosides, Terpenoids & Flavonoids and Tannins in both the extracts whereas reducing sugars and saponins were absent in acetone fruit extract. The phytochemical screening revealed

Table - 1 Phytochemical analysis of both the extracts of *Kigelia Africana*

Plant extracts	Alkaloids	Glycosides	Terpenoids	Flavonoids	Tannins	Reducing Sugars	Saponins
Aqueous extract of *Kigelia africana*	+	+	+	+	+	+	+
Acetone extract of *Kigelia africana*	+	+	+	+	+	-	-

Antioxidant activity of aqueous and acetone extracts of Kigelia Africana

that *Kigelia africana* fruit extract contain alkaloids, flavonoids, tannins, cardiac glycosides, cyanogenic glycosides, anthraquinone glycoside, antho-cyanosides (anthocyanin pigment) and reducing compounds[13]. Terpenoids reduces sugar level in the blood hence *Kigelia Africana* shows anti diabetic activity[14].

Antioxidant activity of aqueous and acetone extracts of *Kigelia Africana*

Enzymatic antioxidants

The levels of Enzymatic Antioxidant assessed in different extracts of *Kigelia africana* were collectively represented in **Table 2.** Among the two extracts of *Kigelia africana*, the highest activity of catalase was observed in aqueous extract (3.267 mg protein) and lowest in acetone extract (1.802 units/mg protein). Peroxidase activity was found to be very high in acetone extract (2.942x106 units/mg protein/min). In plants, antioxidant enzyames namely catalase[15] and peroxidase have been shown to increase when subjected to stress conditions. The ascorbate oxidase activity was highest in aqueous extract (0.0315 units/mg protein) and lowest in acetone extract 0.0123 units/mg protein.

Non-enzymatic antioxidants

The concentration of non enzymatic antioxidant in *Kigelia africana* extracts was also assessed and the results are represented in **Table - 3**. Vitamin C content was low in acetone extract (0.144 mg/ g tissue), whereas high in aqueous extract (0.427 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[16].

Antibacterial activity of aqueous and acetone extracts of *Kigelia africana*

The *in-vitro* antibacterial activity of aqueous and acetone extracts of *Kigelia africana* against *Proteus vulgaris, E.coli* and *Staphylococcus aureus* bacteria strains isolated from the Urine sample of stone patients were examined by Agar well diffusion method and the results are represented in **Table- 4**. It has been found that in case of Gram +ve bacteria aqueous extract of *Kigelia africana* showed maximum antibacterial activity i.e. against *Staphylococcus aureus* (20 mm) while acetone extract of *Kigelia Africana* showed minimum antibacterial activity against *Staphylococcus aureus* (16 mm). In case of Gram -ve bacteria acetone extract of *Kigelia africana* showed minimum antibacterial activity against *E coli* (7 mm) and against *Proteus mirabilis* (6 mm) while aqueous extract of *Kigelia africana* also showed maximum antibacterial activity against *E. coli* (17 mm) and against *Proteus vulgaris* (11mm).

Table - 2 Enzymatic Antioxidant analysis in both the extracts of *Kigelia Africana*

Samples	Catalase μ/moles of H2O2 decomposed/min/g protein	Peroxidase IU/L	Ascorbate oxidase μ mole/ml
Aqueous extract of *Kigelia Africana*	3.267	2.904x106	0.0315
Acetone extract of *Kigelia Africana*	1.802	2.942x106	0.0123
	1 unit = μ/moles of H2O2 decomposed/min/g protein	1 unit = μ moles pyrogallol oxidized/min	1 unit = 0.01 O.D change/min

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

Samples	Vitamin C (mg/g)
Aqueous extract	**0.427**
Acetone extract	**0.144**

Antibacterial activity of aqueous and acetone extracts of *Kigelia africana*

Table- 4 Zone of inhibition of aqueous and acetone extracts of *Kigelia Africana*

Bacterial species	Aqueous extract (mg/ml)	Acetone extract (mg/ml)
Staphylococcus aureus	20 mm	16mm
E.coli	17 mm	7 mm
Proteus vulgaris	11 mm	6 mm

Plants extracts are usually more active against gram positive bacteria than gram negative bacteria[17]. According to Abu-Shanab *et al.,* 18 gram negative bacteria are more resistant to plants extract as compared to gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism and this is in support of the present finding which showed that both the extracts of *Kigelia africana* were effective on the growth of *S.aureus* which is gram positive bacterial species. So from the present study it has been concluded that aqueous extract of *Kigelia Africana* possess significant amount of phytochemicals, antioxidant enzymes and antimicrobial agents than the acetone extract. Further studies are needed to isolate the exact active component which are responsible for antimicrobial activities. It is hoped that this report will serve as a basis of information for future project to be embark on in order to evaluate the potentials of *K. pinnata* (Lam) Benth as a strong medicinal plant in improving human health status.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

1. Graham, J.G,; Quinn, M.L.; Fabricant, D.S. and Farnsworth, N.R. Plants used against cancer– an extension of the work of Jonathan Hartwell. 2000.
2. Singh, M. *International Journal of Drug Research and Technology*. 2012; 2(2): 203-207.
3. Soulsby, J. Resistance to antimicrobials in humans and animals. *Braz. J. Med.* 2005; 331: 1219-1220.
4. Burt, S. Essential oils: their antimicrobial properties and potential application in foods-A review. *International Journal of Food Microbiology*. 2004; 94: 223- 253.
5. Khan, M.; Kihara, M. and Omoloso, A. Antimicrobial activity of the alkaloidal constituents of the root bark of Eupamatia laurina. *Pharmaceut. Biol.* 2003; 41: 277- 280.
6. Aburjai, T.; Darwish, M.; Al- Khalil, S.; Mahajznh, A. and A1- Abbadi, A. Screening of antibiotic resistance inhibitors from local plant materials against two Abu- Shanab B, different strains of Pseudomonas aeruginosa. *J. Ethnopharmacol.* 2001; 76: 39-44.
7. Harborne, J.B. Phytochemical methods, London. Chapman andHall, Ltd. 1973, p. 49-188.
8. Siddiqui, A.A. and Ali, M. Practical Pharmaceutical chemistry. CBS Publishers and Distributors, New Delhi, First Edition. 1997, p. 126- 131.
9. Sinha, A.K. Colorimetric assay of catalase. *Anal Biochem*. 1972; 47:389-394.
10. Addy, S.K. and Goodman, R.N. Polyphenols oxidase and Peroxidase in apple leaves inoculated with a virulent or an avirulent strain for Ervinia amylovor. *Ind phytopath*. 1972; 25: 575-579.
11. Vines, H.M. and Oberbacher, M.F. Response of oxidation and phosphorylation in citrus mitochondria to arsenate. *Nature*. 1965; 206:319-320.
12. Sadasivam, S. and Manickam, A. Vitamins, In Biochemical methods. Eds, A. New Age International (P) Limited, New Delhi, 2nd Eds. 1997, 85-186.
13. Azu, O.O.; Duru, F.I.O.; Osinubi, A.A.; Noronha, C.C.; Elesha, S.O. and Okanlawon, A. Preliminary study on the antioxidant effect of *Kigelia africana* fruit

extract (Bignoniacieae) in male Sprague- Dawley rats. *African Journal of Biotechnology*. 2010; 9 (9):1374-1381.

14. Nyarko, A.K.; Okine, L.K.N.; Wedzi, R.K.; Addo, P.A. and Ofosuhene, M. Subchronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat:absence of organ toxicity and modulation of cytochrome P450.*J Ethnopharmacol.* 2005; 97 (2) 319-325.
15. Hertwig, B.; Steb, P. and Feierabend, J. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant physiol*. 1999; 100:1547-1553.
16. Foyer, C. Ascorbic acid. In: Antioxidants in Higher plants. Eds. Alscher, R.G. and Hess, J.L. CRL Press, Boca Raton. 1993, pp 31-58.
17. Basri, D.F. and Fan, S. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian J. Pharmacol.* 2005; 37(1): 26-29.
18. Adwan, G.; Abu Safiya, D.; Jarrar, N. and Adwan, K. Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turk. J. Biol*. 2004; 28: 99-102.

Phytochemical and Antimicrobial Studies on the Leaves of *Sapium Sebiferum Roxb*

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Abstract- The present study was aimed at detecting the phytochemicals and evaluating antimicrobial activities of ***Sapium Sebiferum Roxb.*** known for its medicinal properties in folk medicine. Phytochemical screening was carried out on the leaves of *Sapium Sebiferum Roxb*.The results show the presence of alkaloids, proteins, carbohydrates, phenolic compounds, tannins, amino acid, fats and fixed oil and saponins The assessment of antifungal activity was performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar PDA) against Aspergillus niger, M.gypseum and T. flavurusclem. The antibacterial effect was studied by the agar direct contact method using B.cereus, B.pumilus, M.luteus and Escherichia coli strains.. These phytochemicals were isolated from the plant with yields of 16.18 % of Petroleum ether extract, 3.51% of Chloroform extract, 23.16 % of Acetone extract and 11.11% of methanol extract. The results revealed that the acetonic extract exhibited significant antimicrobial activity of concentration of 100-500 ì/ml respectively against tested organisms, particularly more effective against B.cerus, B.pumilus and T. flavurusclem than the other extract when compared to the standard drug (chloramphenicol).

Keywords: *Sapium Sebiferum Roxb*, 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 .Fruits are three- lobed, three-valved capsules.[2] Its wood has been used to make various implements, toys, furnitures, inferior quality pencils, cricket bats and Chinese printing blocks.[3] In Chinese medicine, its oil is used as purgative and emetic. Resin from root bark is considered as purgative. The seed is antidote, emetic and purgative.[4]

Material and Methods

Collection and identification of leaves of *Sapium Sebiferum Roxb*

Leaves of *Sapium Sebiferum Roxb* were collected from FRI, Dehradun (India). Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), Botanical Survey of India, Northern regional centre, Dehradun (BSI).

Extraction of leaves of *Sapium Sebiferum Roxb* in different solvents (Non-polar to Polar)

The collected plant material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (500 gm) of ***Sapium Sebiferum Roxb*** were crushed. The crushed leaves were 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.

Phytochemical analysis of different extracts

The different extracts of leaves of ***Sapium Sebiferum Roxb*** were tested for various components:

Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dilute HCl and filtered. The filtrate was then tested for following colour test.

(i) Mayer's test (a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. **(b)** 5 gms of potassium iodide was dissolved in 20 ml of distilled water. **(a)** and **(b)** was mixed and the volume was adjusted

to 100ml with distilled water .Appearance of cream colour precipitate with Mayer's reagents showed the presence of alkaloids.

(ii) Wagner's Test- 1.27 gm of iodine and 2 gm of potassium iodide was dissolved in 5 ml of water and made up the volume to 100ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloids.

(iii) Hager's test- Take 20 ml of saturated solution of picric acid and add few drops of it to 2-3 ml of extract. A yellow color was observed.

Detection for carbohydrates and glycosides

(i) Molisch's test- 10 gm of alpha naphthol was dissolved in 100 ml of 95% alcohol. Extract was treated with this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides of the test tube, purple or violet color appeared at the junction.

(ii) Benedicts test- The test solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed if reducing sugars were present.

(iii) Fehling's Test- 6.932 gm of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 gm of potassium sodium tartarate and 10 gm of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solution was mixed in equal volume prior to use and few drop of sample was added and boiled, a brick red precipitate of cuprous oxide was formed, if reducing sugars were present.

Test for sterols and triterpenoids

Salkowski test: Extract was treated with few drops of conc. Sulfuric acid, shake well and allowed to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

Test for proteins and amino acids

(i) Ninhydrin test- 1gm of ninhydrin (indane1, 2, 3 trione hydrate) was dissolved in n-butanol and make the volume to 100ml. Extract treated with this solution gave violet colour on boiling.

(ii) Biuret test- To 3ml test solution 4% w/v NaOH and few drops of 1% w/v copper sulphate solution were added. A blue color was observed.

Test for sponins

Foam test- 1ml of extract was diluted with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicated the presence of sponins.

Anti-microbial activity of different extracts

The anti-microbial activity of the leaves of ***Sapium Sebiferum Roxb*** was carried out. The leaves extract were screened for anti bacterial and anti fungal activities.

Anti bacterial activity of leaves extract

In this study, the anti bacterial activity was studied against the micro organism and the bacterial cultures used were- (i) B. cerus (ii) B. pumilus (iii) M. luteus (iv) Escherichia coli

These bacterial cultures were maintained on nutrient agar slants at first, being incubated at 37° c for about 18-24 hours and then stored at 4° 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°c overnight. To test anti bacterial activity, the well diffusion method was used.

Culture media preparation

The microbiological media prepared as standard instruction provided by HI-Media Laboratories, Mumbai. The media used for anti-bacterial activity was Muller-Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121°C at 15 psi for 15-30 minutes 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 solidification, 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 asceptic 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 leaf extract from hole through the solidified agar layer of petridish 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 overnight at 37°C ± 0.5°C in the incubator.

Inhibition 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 standard drug.

Results

Table-1 Percentage yield of different extracts *Sapium Sebiferum Roxb leaves*

Sl. No.	Solvent system	%age yield
1.	Petroleum Ether	16.18 %
2.	Chloroform	3.51 %
3.	Acetone	23.16 %
4.	Methanol	11.11 %

Table-2 Qualitative Phytochemical Chemical Analysis of Extract of *Sapium Sebiferum Roxb* leaves

Test performed	Pet. Ether Extract	Chloroform Extract	Methanol Extract
Test for Alkaloids			
Mayer's test	_	_	_
Hager's test	_	_	+
Wagner's test	_	_	+
Test for Carbohydrates			
Fehling test	_	-	+
Molish test	_	_	-
Benedict test	_	-	+
Test for phenolic compounds and Tannins			
Vanillin HCL acid test	_	-	+
Dil. $FeCl_3$.test	_	-	+
Lead Acetate Test	_	+	+
Test for Sterols / Triterpenoids			
Salkowaski test	+	+	
Test for Saponins			
Saponins Test	_	_	+
Test for Proteins and acids			
Ninhydrin test	_	_	-
Biuret test	_	_	_

Key (-) Absence, (+) Presence

Table-3 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		
						Ampicillin	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- 4 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	
						Amphotericin-B	Clotrimazole
1.	*Aspergillus niger*	_	_	-	-	_	11mm
2.	*M. gypseum*	18	14 mm	20 mm	-	_	_
3.	T. flavurusclem	12 mm	12 mm	22 mm	-	_	12mm

Discussion

Phytochemical studies reveal that acetonic extract was the richest extract for phytoconstituents. It contains all tested phytoconstituents as shown in table-2. Acetonic extract showed good antibacterial activity against B. cereus and B. pumilus and good antifungal activity against T. flavurusclem in comparison to the standard drug.

References

1. Radcliffe,A. Notes on African and Madagascan Euphrbiaceae,Kew Buttelin,1997; 52:171-176.
2 Chettri, N. and Sharma, E. Iintrogression and taxonomy of the mountain birch in SW Greenland compared with related results from Iceland and Finnish Lapland. Meddel. Grfnlan, Bioscience, 2000; 3(3):21-29.
3. Duraiswamy, B.; Satishkumar, M.N. et al. Hepatoprptective activity of sepium Bark on D-galactosamine induced Hepatic insult., world journal of pharmacy and pharmaceutical science, 2012; 1(1):456-471.
4. Singh, S. and Yadav, S. Sepium utilis: A Potential Herbal Medicine, International Journal of Pharmaceutical and Biological Archives, 2012; 3(3):493-498.

Quantitative Evaluation of Carbohydrate Levels in Different Vegetables by UV-Visible Spectrophotometer

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Abstract- A study was carried out to determine the soluble carbohydrate content of some selected vegetables which include Carrot, Moringa, Spinach, Sorrel leaves, Curry leaves, Fenugreek leaves, Drumstick leaves and Lettuce. Soluble carbohydrate was determined by Anthrone method Spectrophotometry at wavelength of 750 nm. It was found that curry leaves had the highest percentage of carbohydrate followed by drumstick, while Lettuce and spinach had least percentage.

Key words- Carbohydrates, Vegetables, UV-Visible Spectrophotometer, Wavelength, Anthrone, Absorbance.

Introduction

Carbohydrates are compounds made up of carbon hydrogen and oxygen, thus they are regarded as hydrates of carbon represented as C (H_2O). They act as the primary source of energy which is converted into glucose to generate energy essential for metabolism in every cell of the body (Flitsch SL., et. al, 2003, M. Sarvan Prasad et.al., 2012) and are of special importance as they constitute more than 50% of the dry weight of most plants (Lehninger, 1993). These compounds perform a number of functions ranging from stores of potential energy in animals to source of energy supporting tissues in plants (Dyke, 1960).Polysaccharides serve for the storage of energy (e.g., starch and glycogen), and as structural components (e.g., cellulose in plants and chitin in arthropods) (M.Sarvan Prasad et.al.,2012). The 5- carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD, and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA(2). Carbohydrates are the most abundant bio-molecules on earth; each year photosynthesis converts more than 100 billion metric tonnes of carbondioxide (CO_2) and water (H_2O) into cellulose and other plant products (Herman, 1968). For most people, between 40% and 60% of total calories should come from carbohydrates, preferably from complex carbohydrates (starches) and naturally occurring sugars (Carl A. Burtis et.al.,2000).

This study was aimed at evaluating the soluble carbohydrate content of some selected vegetables consumed locally viz, sorrel (inflorescence), carrot and *Moringa* leaves with a view to determine whether they meet the dietary requirement of consumers. It is envisaged that the findings of the investigation would provide additional information on the nutritional status of the vegetables.

Material and Methods

Instrumentation- Ultraviolet visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Ultraviolet-visible spectroscopy was recorded on ELICO SL-160, India.

Sample preparation

The vegetables used for this investigation were obtained from field to ensure a supply of fresh samples throughout the period of the study. Moringa leaves, carrot, spinach, sorrel leaves, curry leaves, fenugreek leaves, drumstick leaves, mint, coriander, lettuce were taken as samples. A sample of each vegetable was washed and ground to a fine pulp using pestle and mortar.

Chemicals and Reagents

Anthrone, Sulphuric acid were purchased from Merck Specalities pvt.Ltd.

Reagent Preparation

0.2 grams of anthrone was weighed accurately and dissolved in 100 ml sulphuric acid , made by adding

500ml of sulphuric acid to 200 ml of water. The reagent was allowed to stand for 30-40 minutes with occasional shaking until it was perfectly clear.(Trevelyan & Harrison, 1952).

Procedure

One gramme(1g) of macerated sample was placed in 25ml of volumetric flask, 10ml of distilled water was then added and shaken vigorously followed by addition of 15 cm^3 of 52% perchloric acid. This was stirred continuously for 30minutes and the mixture was later filtered using Whatman no1 filter paper. Pipetted out 1ml of each extracted sample into a 25ml of volumetric flask and add 2ml of freshly prepared anthrone reagent in each volumetric flask and finally make up the volume up to the mark with distilled water. Reference was prepared by taking 2ml of anthrone reagent in a 25ml of volumetric flask and made up the volume up to the mark with distilled water. The wave length of the above prepared samples was checked in uv-visible spectrophotometry and wavelength was set at 750nm. At that wavelength the developed colour absorbances were noted for the samples. The total soluble carbohydrate was then estimated using the standard curve of Glucose (Pearson *et al*., 1976).

Results and Discussion

Soluble carbohydrates content of Morigna leaves, carot, Spinach, Sorrel leaves, Curry leaves, Fenugreek leaves, Drumstick leaves and Lettuce is given in the table. It was found that curry leaves contain maximum carbohydrate percentage and Lettuce contain least carbohydrate percentage. The recommendation for the general population is that carbohydrate should supply 50% to 55% of total calories and 130 gm per day (520 calories per day) for male and female adults and for athletes in between 55% to 65 % of total calories.

References

1. Carl, Burtis A.; Ashwood, Edward R. and Norbert, W. Tietz fundamentals of clinical chemistry. 2000.
2. Dyke, S.F. The carbohydrate. Volume V., Interscience publishers, New York, 1960; 120-125.
3. Flitsch, S.L. and Ulijn, R.V. Sugars tied to the spot, *Nature*, 2003; 421, 219–220.
4. Herman, J.D. Commercial vegetable growing. Oxford tropical Hand book. 1968; 129.
5. Lehninger, A. Principles *of* Biochemistry, 3rd edition. Worth Publishers, New York. 1993; 184-185.
6. Prasad Sravan, M.; Madhu, C.H.; Venkateshwalu, G. and Sabath Manoranjan, *Asian J. Pharm. Ana.*, 2012; 2(1),10-11.
7. Pearson, D.; Melon, H.K. and Ronald, S. Chemical analysis of Food, 8th edition. Churchill Livingstone, 1976; 5-63.
8. Trevelyan, W.E. and Harrison, J.S. *Biochem. J.*, 1952; 50, 298.

Table

S.No	Name of Vegetable	Carbohydrate contents (%)
1.	*Moringa leaves*	0.240 %
2.	*carrot*	0.185 %
3.	Spinach	0.073 %
4.	Sorrel leaves	0.155 %
5.	Curry leaves	0.995 %
6.	Fenugreek leaves	0.430 %
7.	Drumstick leaves	0.79%
8.	Lettuce	0.0675%

Aroma Profile and Comparison of Chemical Composition of Thymus Serpyllum From Lower Hill of the Garhwal Himalaya

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Abstract- The Essential oil content in inflorescence of *Thymus serpyllum* cultivated in the hill of Uttarakhand was found to be 0.70 % on dry weight basis. The oil analyzed by capillary GC and GC-MS. Eighteen constituents, representing 98.71% of the oil were identified. The major components of the oil were thymol (36.8%), terpinen-4-ol (21.4%) and limonene (12.60%). The quality of lavender oil produce in Garhwal Himalyan region of India was found to compete with standard quality of oil from other parts of globe.

Keywords- Tymol, *Thymus serpyllum*, essential oil, GC, GC-MS

Introduction

Thymus serpyllum L. is a perennial herb indigenous in central and southern Europe, Africa and Asia. It is widely used in folk medicine in the treatments of variety of diseases such as gastro enteric and bronchopulmonary disorders, as well as due to its anthelmintic, carminative, sedative and diaphoretic properties[1].

Thyme oil is among the world's top 10 essential oils also used as a preservative for food[2]. The aromatic and medicinal properties of the Thymus species have made it one of the most popular herbs. The genus Thymus has numerous species and varieties and their essential oils have been studied earlier[3]. Thyme was used medicinally by the Egyptians, Greeks, and Romans. Most of the present day research has centred on thyme's ability as an antibacterial and anti-infectious agent, even when diffused in the air. There are several species of thyme oil in use, and although the strongest is red thyme and the gentlest is Linaloöl, their uses are the same. The difference is in their relative strength.

Although the essential oil compositions of thyme grown in different parts of the globe have been investigated[4]. But the present study aims to examine the chemical composition of *Thyme* from Garhwal region of Uttarakhand Himalaya and to compare it with previous reports.

Material and Methods

Collection of plant material

The samples of wild growing *Thymus serpyllum* plants were collected during the flowering stage in May 2013 from Kalsi (Dehradun), which is located on the top of foot of lower Shiwalik mountain (Himalaya) at altitude about 700 m (a.s.l). The plants were identified by Dr Sandeep Dhyani, Faculty of Biotechnology, GRDPG IMT, Rajpur Dehradun, Uttarakhand. The samples were dried in shadow at room temperature for 10 days. Voucher specimens where deposited in Herbarium of Botanical Department of BSI, Dehradun.

Isolation of the essential oils

Air-dried aerial parts of *Thymus serpyllum* deprived from wooden parts (100 g) were subjected to hydrodistillation, using Clevenger-type apparatus for 3 h according to the standard procedure. Essential oils thus obtained were dried over Na_2SO_4 and stored in a sealed dark vials, then kept at 4°C prior to further analysis. The essential oil content was determined as percentage on fresh weight basis as average of three independent extractions of each site to minimize error (Percentage yield of essential oil was determined on fresh weight basis which was found by taking average of three extractions). Extracted oils were combined for further analysis.

GC Analysis

Analyses of the oil samples was carried out by using HP7890 GC manufactured by Agilent equipped with a flame ionization detector (FID) and a HP-5 fused silica column (30 m x0.32 mm x0.2ìm film thickness). The

sample was injected directly into the column. The injector and detector temperatures were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed from 60°C to 220°C with an increase in rate of 3°C/min injected 0.2ìl oil.

Gas chromatography-mass spectroscopy (Gc-Ms)

Analysis of the oil was performed out on a Agilent mass spectrometer (Model 5975C) coupled to a Agilent GC with a 60 m x0.32 mm x 0.2ìm film thickness column (DB5). The sample was injected directly split less mode. Helium was used as the carrier gas (flow rate 1ml/min). The oven temperature was programmed from 60°C to 220°C at 3°C/min. Other conditions were other conditions were the same as described under GC. The identity of the constituents of the oils was established on the basis of GC retention indices, by comparing their 70 eV mass spectra with those reported in literature.

Identification of components

The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, C_9-C_{32} under experimental conditions, co-injection with standards (Aldrich and Fluka), MS library search (NIST and WILEY), and by comparing with the MS literature data (Adam 2007). The relative amounts of the individual components were calculated on the basis of GC peak area (FID response) without correction factors.

Result and Discussion

The essential oil content in the dry at room temperature (25- 30°C), inflorescence of *T serpyllum* cultivated in the lower Himalayan tract of Garhwal Himalaya situated in western Himalaya region was found to be 0.55 %.

Our results on chemical profiling of *T. serpyllum* essential oil are in agreement with several other studies (Raal et al., 2004; Verma et al., 2009; Verma et al., 2011;). Eighteen compounds were identified in T. serpyllum oil, which accounted for 98.71% of the total oil; the major components of the oil were thymol (36.8%), terpinen-4-ol (21.4%) and limonene (12.60%). á-terpinyl acetate (5.7%), Delta-3 carene (3.7%), Borneol (3.6%), á-terpinene(2.1%), and á-pinene(1.8%) are among the major terpenoids present in the oil. In a similar study carried out with the *T. serpyllum* oil from Serbia showed highest concentration of Thymol (38.5%), p-cymene (8.9), Borneol(6.0%), and carvacrol (4.7%). However most of the components were mearly in similar concentrations[5].

It means that oil produces in Kalsi Dehradun region compete with international market. The variation (Qualitative and quantitative) could be due to difference in location, elevation, genetic makeup of

Table-1 Essential oil composition of *Thymus serpyllum* from Dehradun

S. N.	RI	Component name	Area %	
			(Kalsi Thyme)	(Serbian Thyme)
1	932	α-pinene	1.8	2.0
2	988	β-myrcene	0.98	1.3
3	1014	α-terpinene	2.1	1.1
4	1408	β-caryophyllene	1.1	1.3
5	1179	*p*-cymen-8-ol	0.56	-
6	1298	carvacrol	0.48	4.7
7	1020	*p*-cymene	0.23	8.9
8	1002	Phellandrene	0.21	-
9	946	Camphene	0.27	2.0
10	1008	Delta-3 carene	3.7	
11	1141	Camphor	0.58	0.7
12	1232	Thymol methyl ether	5.40	-
13	**1289**	**Thymol**	**36.8**	**38.5**
14	**1024**	**Limonene**	**12.6**	**0.6**
15	**1174**	**terpinen-4-ol**	**21.4**	**0.7**
16	1506	α -bisabolene	1.2	-
17	1346	α-terpinyl acetate	5.7	-
18	1165	Borneol	3.6	6.0
19	1093	Linalool	-	2.4
20	1226	Thymol Methyl Ether	-	3.8
21	1050	λ – terpenene	-	7.2
22	883	β –myrcene	-	0.2
23	1190	Cis-dihydrocarvone	-	0.1
24	1426	Aromandendree	-	0.1
25	1566	Spatulol	-	0.3
Total			**98.71**	**99.51**

the plants or due to adaptive process to particular ecological conditions. According to observation of Lawrence difference in quantity of lavender oil components may depend upon plants genotype, cultivation area, altitude and microclimatic features of the habitat[6-7]. A lot still needs to be done in the area of investigating the pharmacological activities of lavender oil. There is a need to identify active compounds in the extracted oil responsible for the various associated pharmacological activities. This will help in specific therapeutic application of the oil. There is also the need for exhaustive characterization of all the compounds in a given oil extract as this will help in a more accurate chemo typing. Chemo typing could be more meaningful and accurate if the sample size and geographic coverage are enlarged while also noting the ecosystem (including soil type, climate/weather condition, season, etc.) and time of collection of cultivation or wild growth. There is the need to also match the various chemo types with the level and type of pharmacologic activities displayed.

References

1. Rustaiyan, A.; Masoudi, S.; Monfared, H.; Kamalinejad, A.; Lajevardi, M.; Sedaghat, T. and Yari, S.; 2000. Volatile constituents of three Thymus species grown wild in Iran. Planta Medica 66, 197.
2. Stahl-Biskup, E. and Saez, F. 2002. Thyme, the Genus Thymus. Taylor and Francis, Lon-don, pp. 331.
3. Guillen, M.D. and Manzanos, M.J. Study of composition of different parts of a Spanish Thymus vulgaris L. plant. Food Chem., 1998; 3, 373–383
4. Adams, R.P. 2009. Identification of Essential Oil Compounds by Gas Chromatography and Mass Spectrometry, fourth ed. Allured Publishing Corporation, Carol Stream, IL, USA.
5. Nikoli, M.; Glamo, J.; Ferreira ICFR. Chemical composition, antimicrobial, antioxidant and antitumor activity of Thymus serpyllum L., Thymus algeriensis Boiss and Reut and Thymus vulgaris L. Essential oils. *Industrial Crops and Products,* 2014; 52. 183– 190.
6. Lawrence, B.M. *Perf. Flav.,* 1994; 19, 33.
7. Lawrence, B.M. (1995-2000). Essential oil, Allured Publishing Carol Stem IL, 2003. ●

In *vitro* Assessment of Antioxidant Activity of Selected Essential Oils

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Abstract- Essential oils are the liquid mixture of volatile compounds obtained by steam distillation from various plants. Many essential oils are known for biological properties. Considering the growing demand of natural antioxidants, three essential oils were tested for their antioxidant activity using DPPH radical scavenging assay and Ferric reducing antioxidant power (FRAP). These oils were also assayed for their total phenolic content. Free radical scavenging activity was found maximum in Thyme oil followed by Eucalyptus oil and Peppermint oil. However in FRAP method, the order of antioxidant power was maximum for Thyme oil followed by Peppermint oil > Eucalyptus oil. A concentration dependent increase in antioxidant activity was also recorded in both assays. Further HPTLC and GC-MS based detection of active compound is needed to understand the mechanism and synergy among various compounds.

Keywords- Essential oil, Antioxidant activity, Polyphenolics, Free radical scavenging.

Introduction

Medicinal plants and their derived products are important therapeutic utility for number of ailments; it has been recorded since ancient times that different parts of medicinal plants are used to cure specific ailments. Owing to wide acceptance for the safety and reliability of these natural entities as compared to expensive and possible adversarial effects comprising synthetic drugs, there is widespread interest in drug derived from plants (Gordon et al., 2001). The custom of indigenous plants in traditional health practices has a long past. Medicinal plants are used to preserve and promote healthy life, prevent disease and cure ailments. It has been assessed that even today, 80% of the world population rely on herbal traditional medicines for their primary health care (Cassady et al., 1990; Cragg et al., 2005; Ahmad et al., 2006).

Aromatic and medicinal plants are the source of natural antioxidants due to their secondary metabolites such as polyphenols. Phenolics can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (Lapornik et al., 2005). Essential oils also called volatile or ethereal oils are aromatic oily liquids obtained from different plant parts and widely used as food flavours (Burt, 2004). The use of essential oils has been well established in food preservation, pharmaceuticals, alternative medicines and natural therapies (Bruckdorfer, 1996; Lis-Balchin and Deans, 1997). Essential oils are complex mixture of terpenes and their compounds. Because of their relatively safe status, essential oils and their constituents are gaining increasing interest, and their exploitation for potential multi-purpose functional use (Ormancey et al., 2001; Sawamura, 2000).

In the recent past, essential oils are reported for their various biological properties such as antiseptic, antifungal, antibiofilm, anti-Quorum Sensing, anti-inflammatory as well antioxidant activities (Ahmad et al., 1998; Ahmad et al., 1999; Beg and Ahmad 2002; Khan and Ahmad 2012; Hussein et al., 2013; Amorati et al., 2014). Further, there is an increase demand for safe antioxidant agents to be used in cosmetics, pharmaceuticals, food preservations and healthcare (Amorati et al., 2014). Though the chemical antioxidants (BHA, BHT etc.) have shown to be toxic in nature (Sasaki et al., 2002), yet the role of essential oil in traditional system of medicine, especially in aromatherapy is well established (Buttner et al., 1996). The search for natural antioxidants has generated interest among scientific community to reinvestigate essential oils as safe and promising antioxidant agent (Wannes et al., 2010; Tongpoothorn et al., 2012; Kapoor et al., 2014). Some reports on essential oil property are contradictory also. Therefore, using more than one method for assessing antioxidant property of essential oil will provide comparative analysis which may be useful in se-

lecting essential oils for different purposes. In this study, we have selected commonly used three essential oils to evaluate their antioxidant activity using two different assays. The polyphenolic content and concentration dependent effect was also determined. Such information is valuable, as essential oils containing high antioxidant activities can prove valuable for maintenance of health and support scientific validation of traditional claims of well-known culinary essential oils.

Table- 1 Physiochemical characteristics of selected essential oil

Name/Common name/Family	Physiochemical Parameters				
	Part used	Colour	Refractive Index	Specific gravity	Solubility in 90% alcohol
Thymus vulgaris (Linn.)/ Thyme oil/Lamiaceae	Seeds	Golden yellow	1.500	0.900	Soluble
Eucalyptus sp (Labill.)/ Eucalyptus oil/Myrtaceae	Leaves	Colourless	1.465	0.940	-do-
Mentha piperata (Linn.)/ Peppermint oil/Lamiaceae	Aerial Part	Colourless	1.463	0.908	-do-

Material and Methods

Collection of essential oil samples

The essential oils were purchased from Hi-media Laboratory Mumbai (Peppermint oil and Eucalyptus oil) and Thyme oil was kindly provided by Fragrance and Flavour Development Center, Kannauj, UP.

Evaluation of physiochemical characteristics of essential oils

Physical and chemical properties of essential oils such as colour, refractive index, specific gravity and solubility in alcohol were determined at Flavour and Fragrance Development Center, Kannauj, India (Table 1).

Total phenolic content

The total phenolic content of the essential oil was determined by the method of Spanos and Wrolstad (1990), as modified by Lister and Wilson (2001). To 0.5 ml of each sample (containing different concentrations (ìg/ml) of oil), 2.5 ml of 1/10 dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45 °C for 15 min. Each experiment was performed in triplicates. The absorbance of all samples was measured at 765 nm using a UV/Vis Spectrophotometer (Spectronic 20 D+, Thermo Scientific, USA). The standard curve of gallic acid is plotted and results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

DPPH radical scavenging assay

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

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

FRAP assay (Ferric reducing antioxidant power assay) Reducing power was measured by the direct reduction of $Fe^{3+}(CN^{-})^{6}$ to $Fe^{2+}(CN^{-})^{6}$, and was determined by measuring absorbance resulted from the formation of the Perl's Prussian blue complex following the addition of excess ferric ions (Fe^{3+}). Hence, the ferric reducing antioxidant power (FRAP) method of Oyaizu (1986) with little modification was adopted to measure the reducing capacity. Different concentrations (µg/ml) of essential oils in 0.75 ml of distilled water were mixed with 1.25 ml of 0.2 M (pH 6.6) sodium phosphate buffer and 1.25 ml of 1% potassium ferricyanide [$K_3Fe(CN_6)$]. After 20 min of incubation at 50 °C for 20 min, the reaction mixture was acidified with 1.25 ml of trichloroacetic acid (10%). Finally, 0.5 ml of $FeCl_3$ (0.1%) was added to this solution, and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicates greater reduction capability.

Results

Total phenolic content

The total phenolics of all the three oils were determined as micrograms of gallic acid equivalents per gram of essential oil (Nakatani et al.,2000; Elizabeth et al.,2007) by computing with standard calibration curve constructed for different concentrations of gallic acid (50ìg to 800ìg). The graph was linear between these concentrations. Thyme oil showed highest amount of total phenolics (388 ± 4.2 mg/g of gallic acid equivalents) while lemon grass oil showed the least (288.89 ± 1.64 mg/g of gallic acid equivalents) as depicted in Table- 2.

Antioxidant activity

A total of three essential oils under study were subjected to antioxidant scrutiny by two standard methods namely DPPH free radical scavenging activity and reducing power activity by FRAP. The oils were ten times diluted in dimethyl sulpho-oxide and were tested at concentration ranging from 100-500 µg/ml. Of these three essential oils, thyme oil demonstrated strong DPPH scavenging activity (>80% decolorization) at 500 µg/ml concentration. The order of activity among these essential oils was maximum for Thyme oil, followed by Eucalyptus oil >peppermint oil as shown in Table- 3. The values were comparable to commercial standards ascorbic acid (94.9%) and BHT (92.8%)

Similarly by ferric reducing antioxidant power (FRAP) method, Thyme oil demonstrated powerful ferric ions (Fe^{3+}) reducing ability; (absorbance e" 1.0). In this assay, the higher absorbance values correspond with higher reducing ability meanwhile moderate ferric ion reducing ability was shown by the two essential oils (Eucalyptus oil and Peppermint oil) when compared to standards (ascorbic acid and BHT). The reducing power of all essential oils increased with increasing concentration of the oil as shown in Table- 4. The comparative analysis of essential oils determined by the two methods suggested, that Thyme oil, possessed promising antioxidant activity whereas Peppermint and Eucalyptus oils possessed relatively low antioxidant activity.

Discussion

The antioxidant potential of essential oils highlights their therapeutic potential both in traditional and modern phytomedicines. Since essential oils consist of terpenes and other different kind of phenolic compound, it would seem reasonable that their mode of action might be related to those of other phenolic compounds (Tassou et al., 2000) and contribute to their antioxidant activity. The hydroxyl groups of phenols donate hydrogen to free radicals and thus inhibit the free radical chain reaction and hence the phenolic contents may contribute directly to the antioxidative action (Chatterjee et al., 2007). Consequently, the antioxidant activities of essential oils are often explained by their total phenolics. In this study, Thyme oil has shown maximum free radical scavenging ability by DPPH method and maximum absorbance by FRAP assay indicating strongest antioxidant property. However, Peppermint oil and Eucalyptus oil have shown moderate antioxidant activity. Our findings are in agreements with reports of various other workers who have investigated for similar antioxidant potential of essential oils and their active compounds from India as well as other parts of the world (Dorman et al., 2000; Manuel et al., 2010; Zahin et al.,2010; Vaibhavi et al., 2010). However, the activities slightly differ due to difference of minor phytoconstituents and the variability in the quantity of major active constituents

which may arise due to different agroclimatic condition, plant varieties and extraction processes.

Conclusion

On the basis of preliminary investigation of the present study, it can be concluded that the above tested oils and their major active compounds have the potential for application in real food system and healthcare.

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Table- 2 Total phenolic concentration as gallic acid equivalents of essential oils.

Essential oil	*Total phenolics (mg/gm of oil)*
Thyme oil	388±4.2
Eucalyptus oil	300±1.94
Peppermint oil	288.89±1.64

Table- 3 Free radical scavenging activity of essential oils by1,1,-Diphenyl picrylhydrazyl method.

Name of the Essential oil	**Concentration of oil (µg/ml)**				
	Percent decolorization				
	100	**200**	**300**	**400**	**500**
Thyme oil	48.02±2.67	55.24±1.78	63.28±1.98	76.60±2.61	80.26±1.67
Eucalyptus oil	16.46±3.23	37.84±3.22	40.79±1.99	47.52±2.99	50.56±2.67
Peppermint oil	13.73±3.01	21.46±1.78	31.64±3.46	37.74±4.23	44.35±1.86
Control (BHT)	87.41±1.21	88.80±1.23	88.90±3.04	89.20±1.25	90.20±2.15

The above data are the mean ±SD of three replicates.
BHT: Butylated hydroxyl Toluene

Table- 4 The ferric reducing antioxidant power of essential oils by FRAP method.

Name of the compound	**Concentration of oil (µg/ml)**				
	Reducing power at Absorbance (700nm)				
	100	**200**	**300**	**400**	**500**
Thyme oil	0.757±0.09	0.793±0.04	0.971±0.01	1.91±0.11	2.05±0.12
Eucalyptus oil	0.31±0.01	0.402±0.03	0.481±0.01	0.719±0.02	0.991±0.07
Peppermint oil	0.185±0.04	0.318±0.03	0.654±0.03	0.981±0.10	1.710±0.06
BHT (Control)	1.321±0.12	1.432±0.06	1.987±0.12	2.122±0.05	2.542±0.32

The above data are the mean ± SE of three replicates.
BHT: Butylated hydroxyl Toluene

References

1. Ahmad, I.; Aqil, F.; Ahmad, F. and Owais, M. (2006). Herbal Medicines: Prospects and Constraints. In: Ahmad, I.; Aqil, F. and Owais, M.(eds) Modern Phytomedicine: Turning Medicinal plants into drugs. Wiley-Germany, pp 59-76.
2. Ahmad, I,; Mehmood, Z. and Mohammad, F. Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol*, 1998; 62: 183-193.
3. Ahmad, I.; Zaiba, Beg A.Z. and Mehmood, Z. Antimicrobial potency of selected medicinal plants with special interest in activity against phytopathogenic fungi. *Indian Vet Med J.*, 1999; 23 (10): 01-08.
4. Beg, A.Z. and Iqbal, Ahmad. In vitro fungitoxicity of essential oil of *Syzygium aromaticum*. *World J Microbiol Biotechnol*, 2002; 18: 317-319.
5. Bruckdorfer, R.K. (1996). Antioxidants, lipoprotein oxidation, and arterial function. *Lipids*, 31: S83-S85.
6. Burt, S. Essential oils: their antibacterial properties and potential applications in foods – a review. *Int J Food Microbiol*, 2004; 94: 223–253.
7. Buttner, M.P.; Willeke, K. and Grinshpun, S.A. (1996). Sampling and analysis of airborne microorganisms. In: Hurst, C.J.; Knudsen, G.R.; McInerney, M.J.; Stezenbach, L.D.; Walter, M.V. (Eds.), Manual of Environmental Microbiology, A.S.M. Press, Washington, DC, pp. 629–640.
8. Cassady, J.M. Natural products as a source of potential cancer chemoterapeutic and chemopreventive agents. *J Nat Prod.*, 1990; 53: 23-41.
9. Chatterjee, S.; Niaz, Z.; Gautam, S.; Adhikari, S.; Variyar, P.S. and Sharma, A. Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*). *Food Chem.*, 2007; 101(2): 515-523.
10. Cragg, G.M. and Newman, D.J. Plants as a source of anti-cancer agents. *J Ethnopharmacol*, 2005; 100 (1-2): 72-79.
11. Dorman, H.J.D.; Figueiredo, A.C.; Barroso, J.G. and Deans, S.G. In vitro evaluation of antioxidant activity of essential oils and their components. *Flav Frag Journal*, 15 (2000), pp. 12–16.
12. Gordon, M.H. (2001). The development of oxidative rancidity in foods, in Antioxidants in Food: Practical Applications, 1st ed., Pokorny, J.; Yanishlieva, N. and Gordon, M., Eds., Woodhead Publishing Limited, Abington, Cambridge, England, pp. 147-158
13. Gyamfi, M.A.; Yonamine, M. and Aniya, Y. Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. *Gen Pharmacol*, 1999; 32(6), 661-667.
14. Husain, F.M.; Ahmad, I.; Asif, M. and Tahseen, Q. Influence of clove oil on certain quorum sensing regulated functions and biofilm of *Pseudomonas aeruginosa* and *Aeromonas hydrophila. J Biosci.*, 2013; 38: 1-10.
15. Kapoor, I.P.S.; Singh, B.; Singh, G.; De Heluani, C.S.; De Lampasona, M.P. and Catalan, C.A. Chemical composition and antioxidant activity of essential oil and oleoresins of nutmeg (*Myristica fragrans* Houtt.) fruits. *Int J Food Prop.*, 2013; 16(5), 1059-1070.
16. Khan, M.S.A. and Ahmad, I. Biofilm inhibition by *Cymbopogon citratus* and *Syzygium aromaticum* essential oils in the strains of *Candida albicans*. *J Ethnopharmacol*, 2012; 140.2 416-423
17. Lapornik, B.; Prosek, M. and Wondra, A.G. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *J Food Eng.*, 2005; 71, 214–222.
18. Lis-Balchin, M. and Deans, S.G. Bioactivity of selected plant essential oils against *Listeria monocytogenes. J Appl Bacteriol.*, 1997; 82, 759–762.
19. Lister, E. and Wilson, P. (2001) Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Crop Research Institute, Lincoln, New Zealand.
20. Manuel, V.M.; Yolanda, R.N.; Elena, S.Z.; Juana, F.L. and José, A. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. *Flavour Fragr J.*, 2009; 25(1):13-19.
21. Ormancey, X.; Sisalli, S. and Coutiere, P. Formulation of essential oils in functional parfumery. *Parfums, Cosmetiques, Actualites,* 2001; 157, 30–40.

22. Oyaizu, M. Studies on product of browning reaction prepared from glucose amine. *Jap J Nutr.,* 1986; 44.
23. Sasaki, Y.F.; Kawaguchi, S.; Kamaya, A.; Ohshita, M.; Kaba sawa, K.; Iwama, K.; Taniguchi, K. and Tsuda, S. The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res.,* 2002; 519, 103–109.
24. Sawamura, M. Aroma and functional properties of Japanese yuzu (*Citrus junos* Tanaka) essential oil. *Aroma Res.,* 2000; 1, 14–19.
25. Spanos, G.A. and Wrolstad, R.E. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *J Agric Food Chem.,* 1990; 38(7), 1565-1571.
26. Tassou, C.; Koutsoumanis, K. and Nychas, G. Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Res Intl.,* 2000; 33:273–80.
27. Tongpoothorn, W.; Chanthai, S.; Sriuttha, M.; Saosang, K. and Ruangviriyachai, C. Bioactive properties and chemical constituents of methanolic extract and its fractions from< i> *Jatropha curcas*</i> oil. *Ind Crop Prod.,* 2012; 36(1), 437-444.
28. Vaibhavi, J.; Rakesh, P.; Pankaj, K.; Neeraj, P.; Sunil, G.; Anupriya, P. and Sonu, S. Cinnamun: a pharmacological review. *J Adv Sci Res.*, 2010; 1(2);19-23
29. Wannes, A.W.; Mhamdi, B.; Sriti, J.; Ben Jemia, M.; Ouchikh, O.; Hamdaoui, G. and Marzouk, B. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. *Food Chem toxicol*, 2010; 48(5), 1362-1370.
30. Zahin, M.; Ahmad, Iqbal and Aqil, F. Antioxidant and antimutagenic activity of *Carum copticum* fruit extracts. *Toxicol In vitro,* 2010; 24(4):1243-49. ●

Enzymatic and Non-Enzymatic Antioxidant Nature of Essential Oils Extracted from Curry Patta , *Pine Apple* and *Chakotra*

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Abstract- In the present study enzymatic and non-enzymatic antioxidant activities of essential oil of Murraya koengii (curry patta), Citrus maxima (chakotra) and Pineapple (Ananas comosus) were studied. Estimation of Antioxidant activity were carried out by b-carotene bleaching method, Thiocyanate assay and Deoxyribose assay. In Thiocyanate assay it was observed that all plant essential oils showed antioxidant activity at low and moderate concentration. The maximum antioxidant nature was shown by Chakotra followed by pine apple and curry patta. In Deoxyribose assay all plant essential oils showed antioxidant activity at lower concentration. Curry patta showed the maximum activity followed by chakotra and pine apple. In b-carotene bleaching method, Chakotra essential oils showed maximum antioxidant activity.

Keywords: antioxidant, enzymatic, non-enzymatic activity, essential oils, Murraya *koengii, Citrus maxima* & Ananas comosus.

Introduction

Essential oils are secondary metabolites which are volatile in nature, insoluble in water, highly soluble in organic solvent, vegetable and minerals oils. These oils consist of terpenes, alcohols, esters, aldehydes, ketones and phenols etc. Citrus fruits are notable for their fragrance or essence, partly due to flavonoids and limonoids contained in the rind, and most of them are juice-laden (German, 1999). The juice contains a high quantity of citric acid giving them their characteristic sharp flavor. They are also good sources of vitamin C and flavonoids (Knight, 1998). Essential oils contain many biochemical properties like antioxidant, anti-microbial, antihistaminic, etc. They are used as flavoring agent or disinfectant or as perfume in food industry, perfume industry or pharma industry.

Citrus fruits are important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez- Lopez *et al.,* 2005; Jayaprakasha and Patil, 2007; Ebrahimzadeh *et al.,* 2004). Flavanones, flavones and flavonols are three types of flavonoids which occur in Citrus fruits (Calabro *et al.,* 2004). The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin (Mouly *et al.,* 1994; Schieber *et al.,* 2001).

Antioxidants are substances that reduce, neutralize, and prevent the damage done to the body by free radicals which are the species containing simply electrons that are no longer attached to atoms. Instead of circling the nucleus of an atom (much like the earth circles the sun), free radicals are both free and radical enough to go careening through our cells (Meister, 1994). A process called oxidation creates free radicals and this process happens in the context of normal metabolic processes and our everyday exposure to our environment. In other words, eating, breathing, and going out in the sun, all contribute to the process of oxidation, free radical formation, and the resulting damage that is caused to the cells of our bodies. It causes the deterioration of bone, joints and connective tissue; the wearing out of organs; the decline of the immune system; the irritating advance of the visible effects of aging; and even, possibly, to some extent, the aging process itself (Padayatty et al., 2003). Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in biochemistry of living organisms (Jeffrey, 1986).

Much like the immune system itself which operates at a cellular level, the hardworking vitamin C reaches every cell of the body. The concentration of vitamin C

in both blood serum and tissues is quite high. In fact, this nutrient plays a major role in the manufacture and defence of our connective tissue, the elaborate matrix that holds the body together. It serves as a primary ingredient of collagen, a glue-like substance that binds cells together to form tissues (Padayatty *et al*., 2003). As a water-soluble antioxidant, vitamin C is in a unique position to "scavenge" aqueous proxy radicals before these destructive substances have a chance to damage the lipids. It works along with vitamin E, a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop free radical chain reactions (Valco *et al*., 2007).

Material and Methods

For experiment Curry Patta, Pineapple and Chakotra were collected from local market of Dehradun. All the fruits were peeled off. After peeling, juice was extracted with the help of juicer and mixer in laboratory. Equal volume of the juices was taken and mixed with 50% ethanol. Centrifuged it at 5000 rpm for 10 minutes. From supernatant, discarded the pellet. Then Protein content was estimated by using Lowry's method and Bradford Method (Sawhney and Singh, 2007).

Enzymatic antioxidant activity: Superoxide Dismutase (SOD) activity assay

In this method four solutions A, B, C and D were prepared.

Solution A: 0.1mM EDTA containing 50mM Na_2CO_3.

Solution B: 90µM Nitroblue tetrazolium in solution A.

Solution C: 0.6% triton X in solution A.

Solution D: 20mM hydroxylamine HCl (pH-6)

To, 1.3 ml of solution A and 0.5ml solution B, 0.1 ml of solution C and 0.1 ml of solution D were added and mixed well. 5µl juice extract was added to that reagent mixture and immediately absorbance at 560nm at interval of 20 second was taken. SOD was calculated.

Estimation of ascorbic acid (Vitamin C) of samples was done by using method given in Introductory Practical Biochemistry book by Sawhney and Singh.

β-Carotene bleaching method

In this procedure, the plant extracts, Vitamin E and BHA (butylated hydroxy anisole) were applied on TLC plates and after developing with a suitable solvent system, plates were sprayed with a betacarotene solution and exposed to daylight until discolouring of the background (6h.) The active compounds were seen as orange colour on the plate. Methanolic extracts of Citrus plants, Vitamin E and BHA were used as positive controls. Extracts which showed strong antioxidant activity were subjected to further tests. The same experiment was performed for the isolated fractions and compounds.

Thiocyanate assay

The peroxy radical scavenging activity was determined by thiocyanate method using Vitamin C as standard. Increasing concentration of the fractions in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04M, pH 7) in a test tube and incubated in darkness at 37°C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentage scavenging inhibitions were calculated and were compared with the standard, Ascorbic acid. A control was also prepared replacing water with plant extract.

Observations

Experimental results of protein content in fruit samples are given in Table-1, Ascorbic acid and SOD activities are given in Table-2. Antioxidant activities by b-carotene bleaching method are given in table-3.

Results and Discussion

From Table- 1, it is observed that for 1ml juice, 3.97 gm of curry patta, 4.17 gm of Chakotra and 4.07 gm of Pine apple were needed. Curry patta was found to have maximum protein content i.e. 125µg/ml, followed by pine apple and chakotra.

Ascorbic acid & SOD in citrus fruits, Superoxide dismutase i.e. enzymatic antioxidant property was found to be maximum in Lemon (18.5unit/mg of protein) followed by chakotra (17.6 unit/mg). Ascorbic acid in the citrus fruits was observed maximum in lemon juice (0.65 mg/ml) followed by chakotra (0.62 mg/ml). Ascorbic acid on the basis of weight of fruit was observed maximum in Chakotra then in lemon

and close result by pine apple followed by mousambi and orange (Table 2).

Pine apple and other citrus fruits contain different chemical compounds like terpenes i.e. limonene is present in lemon which gives there characteristic taste and smell. Lemons contain significant amount of citric acid which provides low pH and sour taste to juice. They also contain vitamin C (ascorbic acid) which is essential to human health. Antioxidant property of citrus fruits is mainly due to vitamin C (ascorbic acid), terpenes like limonene and flavonoids compounds. The results reported by Gavy and Singh also support our findings (Padayatty *et al*., 2003).

β-Carotene bleaching method

Antioxidant activity by b-Carotene Bleaching method was determined by taking 10 and 20 mL of essential oils. Results are given in Table- 3. In this method, Chakotra has shown maximum activity followed by curry patta & pine apple. Similar trend was obtained when concentration of essential oil was increased up to 20 mL. But on very high oil concentration, antioxidant property might vary. Thiocyanate method also showed that Chakotra has maximum antioxidant property, followed by curry patta and pine apple.

References

1. German, J. "Food processing and lipid oxidation". *Adv. Exp. Med. Biol.*, 1999; 459: 23–50
2. Knight, J. "Free radicals: Their history and current status in aging and disease". *Ann Clin Lab Sci.*, 1998; 28 (6): 331-46
3. Jacob, R. "Three eras of vitamin C discovery". *Subcell Biochem.*, 1996; 25: 1–16.
4. Meister, A. "Glutathione-ascorbic acid antioxidant system in animals". *J Biol Chem.* 1994; 269 (13): 9397 – 400.
5. Gaby, S.K. and Singh, V.N. "Vitamin C,"-Vitamin Intake and Health: A Scientific Review.
6. Padayatty, S.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J.; Chen, S.; Corpe, C.; Dutta, A.; Dutta, S. and Levine, M. "Vitamin C as an antioxidant: evaluation of its role in disease prevention". *J Am Coll Nutr.*, 2003; 22 (1): 18 – 33.
7. Jeffrey Bland, Ph.D. The Nutritional Effects of Free Radical Pathology: 1966/A Year in Nutritional Medicine, Keats Publishing Inc., New Canaan, CT, 1986, p. 16.
8. Padayatty, S.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J.; Chen, S.; Corpe, C.; Dutta, A.; Dutta, S. and Levine, M. "Vitamin C as an antioxidant: evaluation of its role in disease prevention". *J Am Coll Nutr.*, 2003; 22 (1): 18 – 35.
9. Valko, M,; Leibfritz, D.; Moncol, J.; Cronin, M.; Mazur, M. And Telser, J. "Free radicals and antioxidants in normal physiological functions and human disease". *Int. J. Biochem. Cell Biol.*, 2007; 39 (1): 44–84.
10. Sawhney, S.K. and Singh, Randhir. 'Introductory practical biochemistry', Fifth Reprint, 2007.

Table- 1 Protein content in samples

S.No.	Samples	Wt. of sample for 1ml of juice (gms)	Protein in Juice Extract µg/ml	Protein in sample µg/gm
1.	Curry Patta	50/12.6 = 3.97	118	118/3.97=29.5
2..	Chakotra	50/12=4.17	115	115/4.17=27.6
3..	Pine apple	50/12.2=4.09	120	120/4.09=29.3

Table- 2 Ascorbic Acid and SOD in citrus fruits

S.No.	Fruit Sample	SOD Activity(unit/mg)	Ascorbic acid mg/ml of juice	Ascorbic acid in per gram of sample
1.	Curry Patta	**18.5**	**0.65**	0.65/5=0.13
2.	Chakotra	17.6	0.62	0.62/4.17=0.14
3.	Pine apple	16.9	0.53	0.53/4.09=0.129

Table- 3 Antioxidant activity by β-carotene bleaching method

S.N	Fruit Sample	Antioxidant activity when Quantity of essential oil -10µL	Antioxidant activity when Quantity of essential oil - 20µL
3	Curry Patta	69.3	72.0
4	Chakotra	70.4	**72.9**
5	Pine apple	68.3	70.0

Screening of Medicinal Plants for Antityphoidal Activity Against Multidrug Resistance in *Salmonella Typhi*

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Abstract- Typhoid is an major public health problem in tropical and subtropical countries including India. Development of multidrug resistance in *Salmonella typhi* is a major problem. There is a great need to develop some new promising natural drugs with high efficacy and lesser side effects as compared to synthetic drugs available in the market for the treatment of typhoid fever. In the present study, four different medicinal plants species,*Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) were screened invitro for their anti-*Salmonella* activity. The aqueous and methanolic extracts of all the four medicinal plants were prepared and the efficacy of these extracts was screened against MDR strains of *S. typhi.* A total of 14 laboratory adopted *S. typhi* strains was used to check the efficacy of aqueous and methanolic extracts of *Ficus benghalensis*, *Ocimum sanctum*, *Ocimum basilicum*and *Mentha arvensis.* Antibiotic susceptibility of all strains were also check to confirm MDR strains of *S. typhi.*It was observed that these strains were found resistant to Trimethoprim, Co-Trimoxazole and Sulfonilamide, and sensitive to Penicillin G, Amikacin, Amoxicillin, Levofloxacin and ciprofloxacin. Furthermore, the aqueous extracts of *Ficus benghalensis, Mentha arvensis* showed maximum inhibitory activity whereas the aqueous extract of *Ocimum sanctum* and *Ocimum basilicum* showed moderate activity against *S. typhi* isolates. While methanolic extracts showed low efficacy against all isolates. The study suggested that the aqueous extracts of *F. behgalensis* and *M. arvensis* have potent antibacterial activity against MDR strains.

Keywords- Anti-salmonella activity, MDR- multidrug resistance, *Salmonella typhi,* aqueous and methanolic extract.

Introduction

Typhoid fever is a worldwide disease primarily found in countries where sanitary conditions are poor. It is considered to be one of the most under-reported diseases in the developing world. In some areas, it has been estimated that typhoid fever is responsible for 2 to 5% of all deaths. *S. typhi*, the etiological agent of typhoid fever, is a gram-negative, flagellated, non-capsulated, non-sporulating, facultative anaerobic bacillus. It has asomatic (O), antigen (oligosaccharide), flagellar (H), antigen (protein) and an envelope (K) antigen (polysaccharide); and has a Lipopolysaccharide macro-molecular complex called endotoxin that forms the outer portion of the cell wall (Zaidi *et al.,* 2003). The multiple drug resistance (MDR) among these bacterial strains is increasing day by day and MDR strains have become more popular. So there is the need to develop new drug based on natural product to overcome this situation. Herbs are the most reliable resources to be studied to discover the chemical entities for health care. Today, there is a widespread demand for drugs derived from plants, and people believe that medicines derived from plants are safe and dependable compared to synthetic drugs that have adverse effects (Ravikumar *et al.,* 2010). Therefore, there is a need to develop alternative antimicrobial medicines from other sources such as plants for treatment of infectious diseases. The present study was planned to screen medicinal plants for antityphoidal activity.

Material and Methods

Microbial strains

Standard of *Salmonella typhi* (MTCC-733)was obtained from IMTECH, Chandigarh and 14 clinical cultures were obtained from the Molecular and Immune-parasitology

research laboratory, Department of Microbiology, Faculty of Biotechnology, Shoolini University, Solan, H.P.

Characterization and Identification of *S. typhi*

Strains were done by using different specific media and compared to standard. All the clinical isolates were identified by biochemical tests.

Plant materials

Fresh parts of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) were used for the study by preparing their Aqueous and methanolic extracts.

Antityphoidal activityassay

All 14 isolates with standard were cultivated in specific media and screened for 8 antibiotics (i.e., Levofloxacin, Amikacin, Amoxicillin, Penicillin G, Trimethoprim, Sulfanilamide, Co-Trimoxazole and Ciprofloxacin) according to Kirby-Bauer method for to identified MDR strain. All MDR and sensitive strains were then screened with aqueous and methanolic extracts of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) by using Agar well diffusion method.

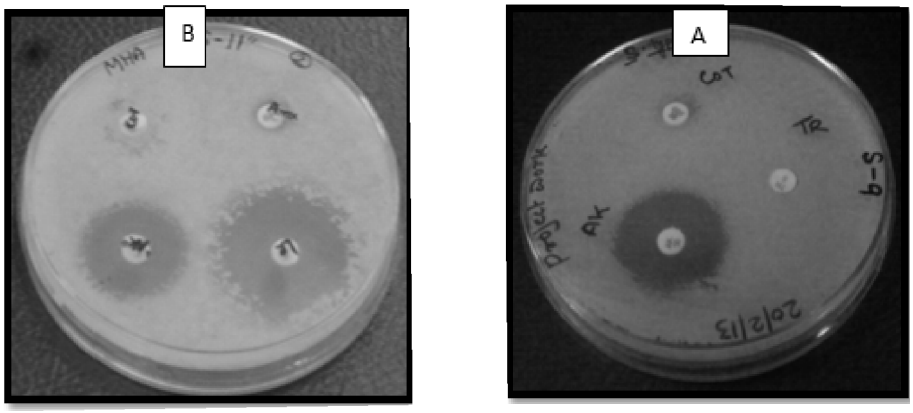

Figure-1 Showing the efficacy of Antibiotics- (A) resistant to Co-trimoxazole, Trimethoprimand sensitive to Amikacin. (B) Resistant to Amoxycillin, Co-trimoxazole and sensitive to Levofloxacin and Amikacin.

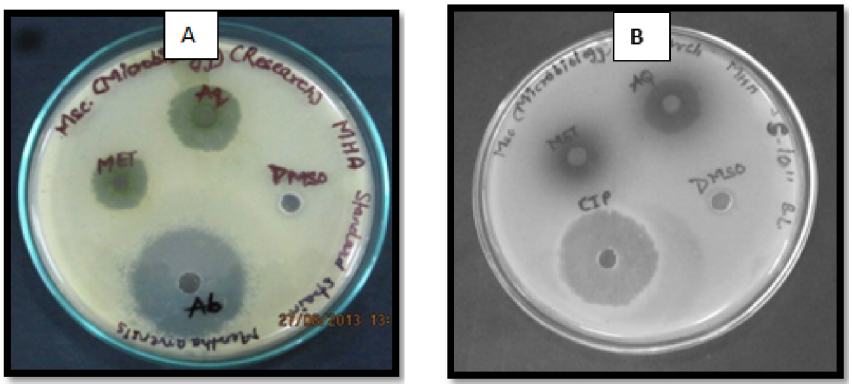

Figure-2 (A) Showing the zone of inhibition against aqueous and methanolic extract of *Mentha arvensis* against *S. typhi* and (B) Showing the zone of inhibition against aqueous and methanolic extract of *Ocimum sanctum*.

Results and Discussion

The biochemical analysis showed that all 14 strains and standard strain is *S. typhi*. The isolates were found highly resistant to Trimethoprim, Co-Trimoxazole and Sulfanilamide, 70% resistant to amoxicillin and 70-100% sensitive for Penicillin G, Amikacin, Amoxicillin, Levofloxacin and ciprofloxacin (Table-1, Fig.-1). All sensitive and MDR isolates were screened with the aqueous and methanolic extract of all four medicinal plants. It was observed that the aqueous extracts showed more efficacy against MDR and sensitive strains of *S. typhi* (Table-2, Fig-2) while methanolic extract of leaf of all plants were found less sensitive and showed very low efficacy against all strains of *S. typhi.*

Typhoid fever is a major public health problem in tropical and subtropical countries including India. The development of MDR strains in causative agent *s. typhi* is an alarming situation in endemic region. To overcome this problem, development of a new drug is an urgent need. The resistance against various drugs has increased due to indiscriminate use of commercially available antimicrobial drugs commonly used for the treatment of infectious diseases. This has led to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. Nearly 80% of the world population depends on the traditional medicine for primary health care, mainly including the use of natural products (Shetty*et al*., 2006).

In the present study, four plants were screened for their antimicrobial activity. The aqueous and methanolic extracts of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) were screened against MDR and sensitive strains of *S. typhi.*We observed that the aqueous extract of *Ficus benghalensis* (Banyan tree), *Ocimum sanctum* (Tulsi), *Ocimum basilicum* (Sweet basil) and *Mentha arvensis* (Pudina) have potent anti *Salmonllla* activity and large zone of inhibhition was observed on agar plates. The methanolic extract of all four plants screened showed no or small zone of inhibitions. This suggested that the methanolic extracts of leaf of all plants do not harbor any molecule which has antibacterial activity while the aqueous extract of leaf harboring the natural molecules has antibacterial activity. Similar to our study, Coutinho et al (2008) reported that the *Mentha arvensis* have potent antibacterial activity and capable of controling MDR strains. The author also observed that the *Mentha arvensis* could serve as a source of plant-derived natural products with antibiotic resistance-modifying activity to be used against multidrug resistant and other pathogens (Coutinho *et al*., 2008).The present study has determined that the aqueous extract of *Mentha arvensis* and *Ocimum sanctum* have high efficacy against the MDR strains of *S. typhi* and able to control the growth of *S. typhi* invitro, which may be used to derive natural molecule. Another fact may be implied that the antimicrobial action of aqueous extract may be due to synergistic action of different phytochemical constituents probably present in the extracts (Bibitha *et al*., 2002).

Table -1 Antibiotic susceptibility analyses for *S. typhi* isolates

Isolates	Zone of Inhibition (in mm)							
	Levofloxacin	Amikacin	Ciprofloxacin	Amoxicillin	Penicillin-G	Co-Trimoxazole	Trimethoprim	Sulfanilamide
S*	17mm	17mm	27mm	18mm	19mm	NZ	NZ	NZ
S-3	30mm	26mm	26mm	15mm	15mm	NZ	NZ	NZ
S-4	36mm	31mm	27mm	17mm	22mm	NZ	NZ	NZ
S-5	19mm	26mm	28mm	29mm	19mm	NZ	NZ	NZ
S-6	30mm	27mm	26mm	10mm	22mm	NZ	NZ	NZ
S-7	28mm	25mm	26mm	9mm	10mm	NZ	NZ	NZ
S-9	28mm	25mm	27mm	26mm	10mm	NZ	NZ	NZ
S-10	28mm	25mm	27mm	9mm	NZ	NZ	NZ	NZ
S-11	33mm	26mm	26mm	10mm	17mm	NZ	NZ	NZ
S-12	26mm	20mm	26mm	17mm	20mm	NZ	NZ	NZ
S-13	29mm	25mm	27mm	17mm	19mm	NZ	NZ	NZ
S-14	32mm	29mm	27mm	18mm	18mm	NZ	NZ	NZ
S-15	25mm	28mm	27mm	17mm	10mm	NZ	NZ	NZ
S-16	30mm	26mm	27mm	9mm	14mm	NZ	NZ	NZ
S-17	36mm	33mm	26mm	34mm	11mm	NZ	NZ	NZ

Where, S*- Standard strain of *S. typhi* (MTCC 733); highly sensitive >25; Sensitive >17; Intermediate 11-16; Resistant <13; NZ- No zone formation; mm- millimeter

Table-2 Antimicrobial activity of methanolic and aqueous extract of different plants against MDR and sensitive isolates of *S.typhi*

Isolates	Zone of Inhibition (in mm)									
	Ficus benghalensis		*Ocimum sanctum*		*Ocimum basilicum*		*Mentha arvensis*		DMSO	Ciprofloxacin
	(AE)	(ME)	(AE)	(ME)	(AE)	(ME)	(AE)	(ME)	-ve control	+ve control
S*	16	8	20	16	14	6	23	20	NZ	27
S-3	15	7	19	16	14	5	22	19	NZ	26
S-4	17	8	20	16	14	5	23	20	NZ	27
S-5	16	7	18	15	13	5	23	20	NZ	28
S-6	16	6	20	14	14	7	22	20	NZ	26
S-7	15	7	20	16	12	5	21	20	NZ	26
S-9	16	8	20	14	14	5	23	19	NZ	27
S-10	16	7	20	16	12	6	23	19	NZ	27
S-11	17	7	19	15	14	5	23	20	NZ	26
S-12	17	6	20	16	14	5	22	19	NZ	26
S-13	16	9	20	16	14	7	22	19	NZ	27
S-14	16	8	20	16	14	6	22	19	NZ	27
S-15	16	7	20	10	12	5	22	19	NZ	27
S-16	16	7	19	15	14	5	23	20	NZ	27
S-17	16	7	19	15	14	5	23	19	NZ	26

Where, NZ- no zone formation; Resistance - < 7; Sensitive - >15; Intermediate 6-7; Highly sensitive- > 20; S*- Standard strain of *S. typhi* (MTCC 733); (AE) –Aqueous extract; (ME) – Methanolic extract

Conclusion

The present study suggested that the antibacterial activity of aqueous leaf extract of selected plant materials against MDR strains of *Salmonella typhi* was observed high determined that these plants are capable in controlling the MDR strains. Further research is required to explore the natural products from these plants to develop new drugs.

References

1. Bibitha, B.; Jisha, V.K. and Salitha, C.V. et *al.* Antibacterial activity of different plant extracts. *Indian J Microbiol.,* 2002; 42:361-363.
2. Centers for Disease Control and Prevention (2005). National Centers for Infectious Diseases, Division for Bacterial and Mycotic Diseases.
3. Coutinho, H.D.; Costa, J.G.; Lima, E.O.; Falcao-Silva, V.S. and Siqueira-Junior, J.P. Enhancement of the Antibiotic Activity against a Multiresistant *Escherichia coli* by *Mentha arvensis* L. and Chlorpromazine. *Chemotherapy,* 2008; 54: 328-330.
4. Crump, J.A.; Youssef, F.G.; Luby, S.P.; Wasfy Rangel, J.M. and Taalat, M. *et al.,* estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerg Infect Dis.*, 2003; 9:539-44.
5. Levine, M.M.; Ferreccio, C.; Cryz, S. and Ortiz, E. Comparison of enteric coated capsules and liquid formulation of Ty 21a typhoid vaccine in randomized controlled field trial. *Lancet.*, 1990; 336:891-4.
6. Ravikumar, S.; Selvan, G.P. and Gracelin, A.A. Antimicrobial Activity of Medicinal Plants along Kanyakumari Coast, Tamil Nadu, India. *African Journal of Basic & Applied Sciences,* 2010; 2:153-157
7. Shetty, S.; Udupa, S.; Udupa, L. and Somayaji, N. Wound healing activity of *Ocimum sanctum* Linn with supportive role of antioxidant enzymes. *Ind Physio.Pharmacol.,* 2006; 50:163-8.
8. Zaidi, A.K.; Hasan, R. and Bhutta, Z.A. Typhoid fever. *N Eng J Med.* 2003; 348:1182-4. ●

Inhibitory Activity of *Ageratum conyzoides* Leaves Extract Against Pathogenic Bacteria

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Abstract- Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. With the continuous use of antibiotics, microbes have become resistant causing clinical problems in the treatment of infectious diseases. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Plant materials remain an important recourse to combat serious diseases in the world. One approach is to screen local medicinal plants and to determine their antimicrobial active compounds. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the therapies involves the use of plant extracts or their active constituents. Keeping in view of the importance of herbs traditionally used for the treatment of infectious diseases, this study is designed to evaluate the antimicrobial activity of *Ageratum conyzoides* used in the Indian system of medicine for the treatment of manifestations caused by micro organisms.

Keywords- *Ageratum conyzoide* leaves extract, pathogenic bacteria.

Introduction

Natural plant products known as herbal medicines have long been used to control microorganisms causing plant and human diseases[1]. Medicinal plants are excellent antimicrobial agents because they possess a variety of chemical constituents that are antimicrobial in nature. Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular plant species[2] because of the need for alternative sources of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics[3].

There is thus continuous effort for synthesis of new chemicals having antimicrobial activity. But most of these chemicals are potentially toxic and are not free of side effects on the Host[4]. This has urged microbiologist for formulation of new antimicrobial agents[5, 6] and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents[7].

The aim, therefore of this work was to evaluate the antibacterial efficacy of *A. conyzoides* Leaves extracts on pathogenic bacterial cultures.

Ageratum conyzoides L. is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities. In Central Africa, it is used to treat pneumonia, but its most common use is to cure wounds and burns. Traditional communities in India use this species as a bactericide, antidysenteric, and antilithic whereas in Asia, South America, and Africa, aqueous extract of this plant is used as a bactericide. In Cameroon and Congo, traditional use is to treat fever, rheumatism, headache, and colic. The whole plant is used as an antidysenteric. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhoea, rheumatism, spasms, or as a tonic.

The leaves extract has been potentially active against bacterial infections, fungal derived skin disease and cancer of cervix, eczema, itchiness of eye and to kill lice. Efficacy of this plant has been determined against II and IV star larvae of *Anapheles stephensi*[8].

Material and Methods

The aerial parts of *Ageratum conyzoides* was collected from from the surroundings of Dehradun city located

in Uttarakhand (India) in April 2014. The plant was properly identified and authenticated.

Method of extraction

The aerial parts of *A. conyzoides* at flowering stage were collected, washed, air dried in shadow and grinded by mixer grinder. After grinding, 300 gm of plant material was 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 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 extra it was stored in air tight container at dark place[9].

Screening for antibacterial and antifungal activity

The antibacterial and antifungal (anticandidal) activity was carried out by employing 24h cultures of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Activity of aqueous and methanolic and Hexane extracts of *Ageratum conyzoides* was tested separately using Agar well diffusion method[10, 11,12,13,14]. The medium was sterilized by autoclaving at 121°C (15 lb/in^2). About 30 ml of the Agar medium with the respective strains of bacteria and fungi was transferred aseptically into 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 24 hrs, antifungal (anticandidal) assay plates were incubated at 28 ±2°C for 48 hrs. The Ciprofloxacin solution was used as a positive control for antibacterial activity, whereas Clotrimazole was used as positive control for antifungal (anticandidal) activity, and diameter of the zone of inhibition was measured.

Results and Discussion

Table- 1 and 2 shows the antibacterial and antifungal (anticandidal) activity of the crude aqueous, Hexane, Acetone and methanolic extracts of *Ageratum conyzoides* on *Staph. aureus, E. coli and Candida albicans*. The methanolic and acetone extract of *A. conyzoides* shows the highest antibacterial activity with the diameter of zone of inhibition ranged 15-20 mm against *Staph. aureus and E. coli.* Hexane and aqueous extract shows the least range with 10-15 mm as the zone of inhibition while no zone of inhibition observed in aqueous extract against *E. coli*. Against *Candida albicans*, methanolic and acetone show the highest zone in the range of 15-20 mm followed by aqueous extract in the range of 10-15 mm while no activity was detected in Hexane extract as depicted in table-2.

The results obtained in this study revealed antimicrobial efficacy of extracts of *A. conyzoides* L. leaves. 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.[15] (2006), but in contrast to the findings of Obi and Onuohau[16], (2000) who documented ethanol as the best solvent for the extraction of plant active substances of medicinal 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 acetone) appeared to be broad spectrum since both the Gram-positive and Gram negative bacteria were sensitive to their inhibitory effects. The choice of these microorganisms used in the work was made due to the fact that some of them are causative agents of intestinal wound and skin infection in human.

Conclusion

It was clearly evident from the study that *A. conyzoides* L. possess antibacterial and antifungal properties. When the antibacterial activity of the extracts of the plant were compared in water, acetone and methanol extracts, significant difference was noticed in their activity. The antibacterial activity of the extracts could be enhanced, if the components are purified. These plants therefore, are potential sources of new drugs for treating infections caused by these clinical pathogens.

Further investigation using bioassay guided fractionation to isolate and characterize the active constituents is under progress.

Antibacterial activity of different extract of *Ageratum conyzoides*

Test organism	Diameter of zone of inhibition (mm)				
	Hexane extract	Methanol extract	Aqueous extract	Acetone extract	Ciprofloxacin
E.coli	1+	2+	NAD	2+	3+
Staph. aureus	1+	2+	1+	2+	3+

*1+; 10-15 mm diameter of zone of inhibition 2+; 15-20 mm 3+; 20-25 mm 4+; Above 25 mm

NAD; No Activity Detected

Antifungal activity of different extracts of *Ageratum conyzoides*

Test organism	Diameter of zone of inhibition(mm)				
	Hexane extract	Methanol extract	Aqueous extract	Acetone extract	Clotrimazole
Candida albicans	ND	2+	1+	2+	3+

*1+; 10-15 mm **diameter of zone of inhibition 2+; 15-20 mm 3+; 20-25 mm 4+; above 25 mm**

References

1. Pattnaik, M.M.; Kar, M. and Sahu, R.K. Bioefficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicum esculentum*. *Asian J. of Pant Sci. and Res.* 2012; 2(2):129-142.
2. Prince, L. and Prabakaram, P. *Asian J. Plant Sci. Res.*, 2012, 1:1-84.
3. Tarun, A.; Rachana, S.; Amar, D.S. and Imran, W. *Asian J. Plant Sci. Res.* 2012, 2(1), 36 – 40.
4. Geddes, A.M. Prescribes' needs for the developed and third world. In the Scientific basis of antimicrobial chemotherapy. Greenwood, FO, O' Grady.Editors. Vol. I Cambridge. Cambridge University Press.1985. P. 265-78.
5. Mamalis, P. Some biological properties associated with aminoxy containing compounds. *Xenobiotica* 1971; 1, 569-71.
6. Baregama, L. and Talesara, G.L. Synthesis and antimicrobial studies of 3 – alkoxy-59p- substituted ary loBiguanidinopentane-2,4 dione and related compounds. *Rese J Chem Enviro*,2002; 6, 59-62.
7. Pandian, M.R.; Banu, G.S. and Kumar, G. A study of the antimicrobial activity of *Alangium salviifolium. Indian JPharmacol.*, 2006; 38, 203-4.
8. Arya, N.S.; Chaurasia, A.; Shakya, M.; Bharti and Sahai, N. Efficacy of *Ageratum conyzoides* against the control of mosquitoes. *IJPSR* (2011); 2(12):3235-3237.
9. Ruedeekorn, W.; Attawadee, S.Y.; Jiraporn, P.; Chitchamai, O. and Arunporn, I. Development and evaluation of granule and emulsifiable concentrate formulations containing derris elliptica extract for crop pest control. *JAFC*. (2009); 57:11234-11241.

10. Perez, C.; Paul, M. and Bazerque, P. Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp*, 1990, 15, 113-115.
11. Murry, P.R.; Baron, E.J.; Pfaller, M.A.; Tenover, F.C. and Yolken, H.R. Manual of clinical microbiology, 6th edition. ASM Press, Washington, DC, 1995, 15-18.
12. Olurinola, P.F. A Laboratory manual of Pharmaceutical Microbiology. Idu, Abuja, Nigeria,1996, 69-105.
13. Srinivasan, D.; Sangeetha, Nathan, Suresh, T. and Lakshmanaperumalsamy, P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol*., 2001, 74,217-220.
14. Greenwood, D.; Slack, R.C.B. and Peutherer, J.F. Microbiology, 14thedition, Churchill andLivingstone, Spencerswood, 1992,pp 1.
15. Ijeh, I. I.; Omodamiro, O. D. and Nwanna, I. J. *Afr. J. Biotech*., **2005**, 4, 953-6.
16. Obi, V. I.; Onuoha, C.; In: Ogbulie, J.N. and Ojiako, O.A. (eds.). Biological and Agricultural Techniques bsmedia Publishers, Owerri, 2000.

Phytochemical Analysis and Antioxidant Activity of *Glycyrrhiza glabara Linn.*

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Abstract- The Licorice plant (*Glycyrrhiza glabara*, family leguminosae), also known as "sweet roots" (contains a compound 50 times sweeter than sugar) and is therefore used in confectionary industry and also finds its application in pharmaceutical industry too; this is attributed to a credible biologically active root component Glycyrrhizin. A study was conducted to evaluate preliminary phytochemical studies and explore the free radical scavenging activity of licorice root extract. Extracts show the presence of various phytochemicals. The methanolic extract shows significant antioxidant activity.

Keywords-*Glycyrrhiza glabara,* Phytochemical analysis, Antioxidant activity.

Introduction

Since the origin of Human life, plants continue to play a therapeutic role to improve Human health. They have been used throughout the world as drugs and remedies for various diseases. Licorice (or liqourice) is a plant of ancient origin and steeped in history. It grows in subtropical climates in Europe, the Middle East, and Western Asia. Licorice extracts and its principal component Glycyrrhizin has extensive uses in food, tobacco products and snuff and in traditional herbal medicines. It is a perennial herb which possesses sweet taste and is, therefore used as a flavouring agent in ayurvedic medicines to disguise the unpleasant flavour of other medications (Biondi et al., 2005). Usually in traditional system of medicine, roots and rhizomes of *Glycyrrhiza glabara* are used. These have extensive pharmacological properties. It contains triterpene, saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances. Hispaglabridins A and B have significant antioxidant activity and both the glabridin and glabrene possess estrogen like activity.

Licorice has been shown to have great antioxidant free radical scavenging (*Haraguchi et al., 1998; Di Mambro & Fonseca, 2005*) and anticonvulsant activities (*Nassiri-Asl et al., 2007*).

It has been shown to decrease circulating levels of testosterone in men *(M.M Rafi et al 2002, D Armanini et al 2002)*. The effect of Glycyrrhizin(GR) on HIV replication in cultures of peripheral blood mononuclear cells (PBMS) from HIV infected patients has also been investigated. The antioxidant and antimicrobial constituents of licorice were studied by some researchers. The antimicrobial Flavanones from leaves of licorice were studied by *Fukui, H..K, et al, and Li.WY*.

Material and Methods

Plant material- *Glycyrrhiza glabara* was collected from the garden of Himalaya Drug Company, Dehradun where medicinal herbs are cultivated.

Extraction

The air dried material (150gm) was finely pulverized and extracted by percolation with water, MeOH (methanol) and hexane for one week at room temperature. The combined extracts were filtered and concentrated using Soxhlet apparatus to obtain crude extract of about 10 gm each of water, methanol and hexane.

Phytochemical screening

The extracts were analyzed for the presence of various phytochemicals such as Tannins, Flavonoids, Saponins, Steroids, Terpenoids, Alkaloids, Glycosides, Proteins. The following tests were performed. (Siddiqui and Ali, 1997).

Tannins

Ferric chloride test- Extract was treated with ferric chloride; appearance of blue colour indicated hydrolysable tannins and green colour condensed tannins.

Flavonoids

Alkaline reagent test- To the test solution few drops of sodium hydroxide solution(1ml 10% NaOH to 3ml extract) was added, intense yellow colour was formed which turned to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Saponins

Froth formation test- 2ml solution of extract was placed in water in a test tube; on shaking stable foam was formed.

Steroids

Salkowski test- Treated the extract with few drops of concentrated sulphuric acid (about 5 drops to 1 ml), red colour at lower layer indicated presence of triterpenoids.

Triterpenoids

Salkowski test- Extract was treated with few drops of concentrated sulphuric acid; formation of yellow coloured, lower layer indicated the presence of triterpenoids.

Alkaloids

1 ml of 1% HCl was added to 3ml extract in a test tube. The mixture was heated for 20 mins, cooled and filtered. About 2 drops of Mayer's reagent to 1ml of extract was added. A creamy precipitate formation indicated presence of alkaloids.

Antioxidant activity

The antioxidant activity (free radical scavenging activity of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Brain-Williams et al. (1995).

The ability of plant extract to scavenge DPPH radical was calculated by the equation-

$$\%age = \frac{(\text{Abs. of blank})-(\text{Abs. of sample})}{(\text{Abs. of blank})} \times 100$$