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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SOME MEDICINAL PLANTS

P.RajaRao[1], T.Sheethal[2], P.Sahithi[3], P M Sameera[4]

[1,2,3]*University College of Technology, Osmania University, Hyderabad (India)*

[4]*S N V Pharma MahaVidyalaya, White House, Taranaka, Secunderabad (India)*

ABSTRACT

The use of medicinal plants for curing diseases has been documented in the history of civilizations. The medicinal plants comprise approximately 8000 species. The medicinal plants are being used to prevent, promote and to cure diseases in recent years. Secondary metabolites that are phytochemicals with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. There has been a growing interest in therapeutic use of nature products. Plants contain not only essential oils but also the anti microbial compounds which are of interest because of their antibiotic resistance. In the present study, plant extracts were taken out in the form of aqueous methanol extractions. Phytochemical screening was conducted to find out the presence of active chemical constituents such as alkaloids, glycosides, terpinoids and steroids. The flavonoids, reducing sugars and tannins were also studied. The species, AzadirachtaIndica, Catharanthusroseus, Lantana camaraand Ficusreligiosa were selected. Different pharmacological studies proved that the high medicinal properties of these plants which are being used in the treatment of diseases. All the plants extracts had shown a significant antimicrobial, phytochemical and anti oxidant activities. The qualitative and quantitative analysis of phytochemicals was carried out by reverse phase high performance liquid chromatography.

Keywords : Phytochemicals , Antioxidant Activities , RP-HPLC

I. INTRODUCTION

In India over one and half million medical practitioners use medicinal plants in preventive, promotive and curative applications. In recent years, secondary plant metabolites (phytochemicals) with unknown pharmacological activities have been extensively investigated as a source of medicinal agents[1] In the last one decade there has been a growing interest to search for phytochemicals of native and wild plants for pharmaceuticals and nutritional requirements. The plant extracts are used as alternative remedies for many infectious diseases. The ingredients found in plants are of interest because they possess antibiotic resistances. Many herbs have been reported to exhibit anti oxidant activities due to the presence of isoflavones, flavonoides and anthocyanin. Anti oxidant based drug formulations are used for the prevention and treatment of depression, hypertension and alziemers disease.

The medicinal plants find application in the pharmaceutical, cosmetic, agricultural and other allied fields. Hypericum perforation is found to be a reputed remedy for depression and ginkgo bioloba is used to treat

tinnitus. Plants like Heliotropiumindicum, Daphnemezereum have shown significant inhibiting effects on tumors. Momordicacharantia (chirantin), Gymnemasylvestre (gymnemic acid) are some medicinal herbs that have shown effectiveness in non insulin dependent diabetics. Commiphoramukul (guggulsterones), Boswelliaserrata (boswellic acid), Withaniasomnifera (withanolides), Ruscusaculeatus (ruscogenin), Harpagophytumprocumbens (harpagoside) are prominent plants which possess anti arthriticactivity. Croton sublyratus (plaunotol) has potential and wide spectrum of anti peptic ulcer property. Bacopamonnieri contains bacosides A and B and bacoside A is a strong antioxidant, which reduces several steps of free radical damage. Coleus forskohlii (forskolin), Grape seed (proanthocyanidins), Camellia sinensis (polyphenols), Huperziaserrata (huperzine), Pinus maritime (Pycnogenol), Borago officinalis (gamma linoleic acid) and vinca minor (Vinpocetine) are potential antioxidants.[2]

1.1 Medicinal plants used in the work

Neem (Azadirachtaindica) : The decoction of the bark is used as a tonic. It is also used in the treatment of filariasis, fever, diabetes, gingivitis, jaundice and syphilis.

Catharanthusroseus :The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. The alkaloids of the plant have tranquilizing properties.

Lantana camara: It prevents absorption of phosphate from food in stomach. It also reduces phosphate levels in the blood in patients with kidney disease.

Triticumaestivum (wheat grass): It helps in increasing production of hemoglobin, prevents tooth decay and reduces bacterial infections. It removes toxins from lever and blood.

Psidiumguajava : The flowers of guava are used in treating bronchitis. The leaf extract is used to treat digestive disorders. The quercetin in guava leaves control the gastrointestinal problems.

Ficusreligiosa : It is used in the treatment of diabetes and high blood pressure. It is also used to reduce migraines, skin diseases and pneumonia. It is helpful in improving blood circulation.

II. REVIEW OF LITERATURE

The use of medicinal plants as a source for relief from illness can be traced back to over five millennia, the written documents of the early civilization in China and India are proved it is doubtless an art as old as mankind. Considering the vast potentiality of plants as sources for antimicrobial drugs systematic investigations were undertaken to screen the local flora for antibacterial and antifungal activity from Ficus,Wheat grass, Lantanum,Neem, Guava and Vinca rosea [3]Different methods are to be used for assessment of antimicrobial activity. Two most commonly used methods are the disc diffusion method (Rios et al., 1988; Pelttari et al., 2002; Khan et al., 2009), and the broth dilution assay (Okusa et al., 2007). The Folin-Ciocalteu procedure was used to assess the total phenolic concentrations of the extracts as Gallic acid equivalents. (V. Nagavani and T. Raghava Rao, 2010)[4]. A significant number of workers have reported regarding the phenolic content and antioxidant activity of various medicinal plants. Antioxidant with remarkable antioxidant activity has been found in many medicinal plants, methanolic extract of mucuna pruriens (seeds) (Rajeshwar et al., 2005),

Thapsia garganica, Artemisia compestris, Anthemis arvensis, Artemisa herba, Teucrium polium, Ruta Montana (Djeridane et al., 2006), Rheum Ribes (Ozturk et al., 2007), Cyperus rotundus (Nagulendran et al., 2007), Equisetum arvense L. (Dukic et al., 2008), Morinda Lucida (Ogunlana et al., 2008), aerial parts of Teucrium polium (Sharififar et al., 2009), Galla chinensis (Tian et al., 2009), Bark of Terminalia arjuna (Shridhar and Gopal 2009) and Egg plant (Singh et al., 2009). It is very interesting that the levels of enzymatic antioxidants were found to be high in the aqueous extracts of fresh flowers of M. champaca. (V. Nagavani and T. Raghava Rao, 2010).UV-visible detectors are best choice to detect the peaks of phenolics (Oliveira et al., 2001; Vinha et al., 2002; Lachman et al., 2003; Eremina et al., 2004; Gudej and Tomcyzk 2004; Prestos et al., 2006; Mozetic et al., 2006; Markowski and Plocharski 2006; Ozkan and Baydar 2006; Dvorakova et al., 2007; Olszewska, 2007; Chunsriimyatav et al., 2009). Available literature indicates that medicinal plants are rich sources of bio pesticide. The use of synthetic chemical insecticides is either not permitted or used restrictively because of the residue problem and health risks to consumers.

III. MATERIALS AND METHODS

Collection of Plant Material and Extraction: Fresh plant or plant parts were collected randomly from the Hyderabad, India. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Aqueous Extraction: Ten grams of dried powder was taken into distilled water and extracted for 6 hours at low heat. Filtration was done for every 2 hrs, through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected. This process was repeated twice and after 6 hrs, the supernatant was concentrated to make the final volume to one-fourth of the original volume. Then the final supernatant was autoclaved at 121ºC, 15 lbs pressure for 15 minutes and then stored at 4 ºC.

Methanol Extraction: Air dried powder of 10g was taken in a conical flask containing 100 ml of methanol plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 h. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of its original volume.

Ethanol Extraction: Ten gram of sample was soaked in 100ml of 95% ethanol and kept at room temperature for 24hours. Then the extract solution was filtered through a Whatmann No.4 filter paper. Finally, the solvent was removed from the sample using a rotary vacuum evaporator until it reaches one-fourth of its volume.

PHYTOCHEMICAL SCREENING: The tests were conducted to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids. The flavonoids,[5] reducing sugars and tannins were also analysed in the ethanol extracts.

FLAVONOIDS DETERMINATION:

Extraction of Flavonoids: One gram crude sample is extracted with 10ml methanol for 5 minutes on a water bath at 60° C and then filtered. This rapid method extracts both lipophilic and hydrophilic flavonoids.

Enrichment : A total of 5ml of methanolic extract is concentrated to about 2ml; 1ml water and 10ml ethyl acetate are added and shaken for several times. The ethyl acetate fraction is separated and reduced to a volume of 1ml.

Phenols Determination: A dilute methanol extract of the plant leaf (0.5 ml of 1:10 gram ml-1) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 ratio diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M). The mixtures were kept for 15 min and the total phenols were determined by colorimetry at 750 nm. Total phenol values were expressed in terms of gallic acid equivalent (mg g –1 of dry mass), which is a common reference compound.

IV. ANTIMICROBIAL ACTIVITY

Growth and Maintenance of Test Microorganism for Antimicrobial Studies: Bacterial cultures of, Escherichia coli (E. coli)Pseudomonas sp, Staphylococcus sp and Salmonella sp and fungal cultures of Aspergillus sp, Mucor sp, Alternaria sp and Fusarium sp were obtained from the Microbiology Department, Acharya.N.G.Ranga agricultural University, Hyderabad. [6]These organisms were used to test antimicrobial activity of plant extracts. The bacterial cultures were maintained on nutrient broth (NB) at 37°C and fungus were maintained on Potato dextrose agar (PDA) at 28°C.

Antifungal Activity: The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg mlG1 concentrations of the extracts were placed on test organism-seeded plates. Solvents which are used in the extract were completely evaporated before application on test organism-seeded plates. Blank disc was impregnated with solvent followed by drying off was used as control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

V. ANTIOXIDANT ACTIVITY

Reducing power assay: Principle- The reducing power of ethonal , methanol and aqeuous plant extracts were determined by the slight modification of the method of Oyaizu [7]. Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Phosphate buffer preparation: Dibasic sodium phosphate (37.50ml of 0.2M) is mixed with 62.5ml monobasic sodium phosphate and diluted to 100 ml with water.

$C_6N_6FeK_3 + FeCl_3$ ----------> $C_6N_6FeK_4 + FeCl_2$

Preparation of standard solution: 3mg of ascorbic acid dissolved in 3ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 10, 25, 50, 75 and 100 µg/ml.

Preparation of test sample: Stock solutions of samples were prepared by dissolving 10mg of dried ethanol extract in 10ml of ethanol to give concentration of 1mg/ml. then prepares sample concentrations of 10, 25, 50, 75 and 100 µg/ml.

VI. IDENTIFICATION OF POLYPHENOL COMPOUNDS BY RP-HPLC METHOD

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry, with the purpose of identifying, quantifying and purifying the individual components of the mixture. The identification was done by RP-HPLC according to a modified method described by Sharma, et al. (2005). Extracted samples (Methanol extract) were filtered through a 0.45µm PTFE syringe tip filter, using a 20µl sample loop. The sample was analyzed using an RP - HPLC system equipped with a waters UV-Visible tuneable detector on a reverse phase (RP C18) column Alltech Interstsil ODS-5µm x 4.6 mm x 150 mm. The flow rate was set at 1ml / minute at room temperature. To perform this study a gradient of three mobile phases was used.

VII. REAGENTS USED

Solvent A:50 mM ammonium phosphate (NH 4H 2PO4) pH 2.6 (adjusted with phosphoric acid)
Solvent B: (80: 20 (v/v)) acetonitrile / solvent A
Solvent C:200 mM of phosphoric acid pH 1.5 (pH adjusted with ammonium hydroxide).

Column Preparation: A fresh, clean column was taken and was filled with the required gradient material. The gradient material used here in this experiment was cotton, silica gel and activated charcoal. The column is first fitted with cotton and then silica gel, activated charcoal and again silica gel were filled in the ratio of 1:2:1.After this again cotton was fitted on the top of the column.

Extraction of the compound: The crude extract obtained was poured in to the column. Then the compound was obtained in the Petri dish which was placed at the bottom of the column in the form of fine powder. This is repeated until required amount of the compound was obtained.

Toxicity bioassay: The column cleanup compounds of the five plants were mixed with 250 g of maize in plastic jars at five different dosages (1.0, 2.5, 5.0, 7.5 and 10.0% w/w).) .Untreated maize was used as control trials were used. Forty unsexed five to eight-day-old Tribolium castaneum adults were placed into the jars arranged in a completely randomized design (CRD) with four replicates. The top of each jar was covered with nylon mesh held tightly with elastic bands. A 2-cm-wide band of fluon (polytetrafluoroethylene) was smeared around the inside near the top of the jar to deter the insects from climbing out. The number of dead insects in each jar was counted every day for the first 4 days. The percent mortality was calculated by expressing the number of dead as a percent of the total number of adult insects introduced into the jar at the start of the experiment. All adult insects were removed from the jars after 4 days.

VIII. RESULTS AND DISCUSSION

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, reducing sugar, flavonoids and alkaloids were present in the samples. The result of the phytochemical screening and analysis shows that these selected plants are rich in at least one of alkaloids, flavonoids, terpenoids, reducing sugars and phlobatannins. The phytochemical

screening shows for the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannins in the plant extracts.

The preliminary screening of plant extracts revealed the presence of bioactive phyto compounds. From the phytochemical screening, Alkaloids, Terpenoids, Flavonoids and Tannins were found in the ethanolic extract of Ficus leaf. Flavonoids were present in all the plant extracts. Wheat grass and Neem had terpenoids, tannins and glycosides. Screening of Lantana revealed the presence of all the phytocompounds tested except alkaloids. Guava leaf showed the presence of alkaloids, terpenoids and glycosides, whereas catharanthus roseus leaf had terpenoids and tannins. Each of the plant extracts (0.5 ml of 1:10 g ml-1) in ethanol was used for flavonoids determination by aluminum chloride colorimetric method and the total flavonoid content in the plant leaf extract was detected from the standard Quercetin graph and standard graph of quercetin is constructed from the values obtained.

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process. The flavonoids contents of the extracts in terms of quercetin equivalent were between 52-286 µg/100µl and the standard graph of quercetin in represented in fig.1.The flavonoid contents in the extracts of Guava (286µg/100µl) were the highest, followed by Ficus (195 µg/100µl) and Lantana(185 µg/100µl).The least flavonoid content was found in Neem(52 µg/100µl),whereas catharanthus roseus and Wheat grass had intermediate values of 142 and 166 µg/100µl respectively and the total phenolic content was found highest in Ficus 34.5 µg/100µl.

The results obtained in the present study revealed that the six medicinal plant extracts ,when tested posses potential antibacterial activity against *Escherichia coli* (*E. coli*), *Pseudomonas sp*, *Staphylococcus sp* and *Salmonella sp* and antifungal activity against *Aspergillus sp*, *Mucor sp*, *Alternaria sp* and *Fusarium sp*. The highest activity was found in methanol extract against E.coli In the case of guava no activity was observed in ethanol & methanol extracts against Salmonella sp. maximum activity recorded was 27mm against Staphylococcus sp. in water extract and minimum activity 6mm against E.coli & Salmonella sp. Catharanthus roseus exhibited anti bacterial activity against E.coli & Staphylococcus sp. in all three extracts maximum being 15mm against E.coli.

Considering the zone of inhibition of the antifungal activity was comparatively lesser than antibacterial. Ficus aqueous and ethanol extracts showed activity only against *Alternaria sp* only. Methanol extract exhibited activity against all fungi tested. The highest being 8mm against *Alternaria*. Wheat grass leaf extract showed highest activity against *Aspergillus* sp.(9mm). Lantana had better antifungal activity.

Qualitative and quantitative analysis of poplyphenolic compounds: The corresponding RP-HPLC chromatogram of methanol extract of Ficus religiosa and guava were identified and quantified on the basis of their retention time values and UV spectra by comparison with those of the single compound in the standard solution. The retention time and the concentration of polyphenolic compounds contained in the extracts are reported in the table 6.There were numerous peaks that were not identified because of lack of suitable standards. The samples were analyzed at least four replications at 280 and 320 nm. The formula to calculate concentration is Concentration = (Sample Area / Standard Area) * (Standard Concn / Sample Concn)

The results show the most important compounds identified in ethanol extract are caffeic acid, p-coumaric acid, rutin and quercetin.The maximum concentration was found in rutin (1.68mg/ml) and the least in

quercetin(0.82mg/ml).. Phenolic compounds have received much attention as one of the principle antioxidants found in plants. Few researchers reported that some essential oils and organic solvent extracts from plants posses antioxidants.

The data in table 7 clearly indicate that the most important compounds identified in methanol extract are kaempferol, rutin and quercetin. The maximum concentration was found in Rutin (1.68mg/ml). Phenolic compounds have received much attention as one of the principle antioxidants found in plants. (Fig 6,7,8).The results in the present study revealed that the two medicinal plant extracts, possess potential antibacterial activity against Escherichia coli (E. coli), bacillus subtilis.

Antibacterial activity: When tested by the disc diffusion method, the ethanol leaf extracts of *catharanthus roseus* showed good activity against *E. coli.* The maximum/optimum antibacterial activity of 26 mm was recorded in ethanol extract of catharanthus roseus. Ethanol extract of neem did not show any activity against *E.coli.* The maximum activity was shown in ethanol extract of catharanthus roseus against (24 mm) Bacillus subtilis and minimum activity with neem extract.

IX. CONCLUSION

From the above research work it can be concluded that the selected plant extracts of the species *Azadirachta Indica, Catharanthus roseus and Lantana camaraand Ficusreligiosa* have shown a significant antimicrobial, phytochemical and antioxidant activities and potential to act as biopesticides. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.[8] The use of these plant extracts also have proven benefits in the treatment of various diseases. The obtained results indicate that the antibacterial activity of vinca rosea is almost equal to gentamycin and increase in concentrations of extract increases the zone of inhibition.. Thus, these extracts may be of great interest for future studies about treatment of many diseases, considering their potent antioxidant activity and low toxicity. However the commercial production is still far away due to the lack of optimization of cultural conditions.

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Table 1: **Phytochemical screening of plant leaf extracts**

Name of the plants	Alkaloids	Terpenoids	Tannins	Reducing sugar	Glycosides	Flavanoids
Ficus	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	+ve
Wheat grass	**-ve**	**+ve**	**+ve**	**-ve**	**+ve**	+ve
Lantana	**-ve**	**+ve**	**+ve**	**+ve**	**+ve**	+ve
Neem	**-ve**	**+ve**	**+ve**	**-ve**	**+ve**	+ve
Guava	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**	+ve
catharanthus roseus	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	+ve

Table 2: Concentrations of Quercetin and absorbance at 765nm

Standard quercitin(µg/100µl)	Absorbance at 765 nm
40	0.348
80	0.532
120	0.737
160	0.964
200	1.161
240	1.372
280	1.574
320	1.782

Table 3: Antibacterial activity of some medicinal plant extracts against bacterial species tested by disc diffusion assay

Name of Bacteria	Zone of inhibition (mm)																	
	Ficus			Wheat grass			Lantana			Neem			Guava			Catharanthus roseus		
	W	ET	MT	W	ET	MT	W	ET	MT	W	ET	MT	W	ET	MT	W	ET	MT
E.Coli	7	9	7	---	12	16	14	6	13	9	6	11	7	6	8	6	15	6
Staplylococcus	13	8	29	7	8	12	13	6	9	6	6	8	27	8	7	13	6	6
Pseudomonas	13	8	9	11	20	15	24	6	7	6	6	6	10	7	9	6	--	9
Salmonella	7	6	9	-	18	9	11	6	7	6	6	7	6	--	--	--	6	--

W—water extract ET----ethanol extract MT---methanol extract

Table 4 : Zone of inhibition against *E. coli*.

Antimicrobial agent	Plate1(15 µl)	Plate2(10 µl)
Ampicillin	34mm	34mm
Gentamycin	30mm	30mm
Neem	0mm	0mm
Catharanthus roseus	26mm	24mm

Table 5: Zone of inhibition against bascillus subtillus

Antimicrobial agent	Plate1(15 µl)	Plate 2 (10 µl)
Ampicillin	30mm	30mm
Gentamycin	28mm	26mm
Neem	10mm	7mm
Catharanthus roseus	24mm	23mm

Table 6 : Polyphenol compounds identified in Ficus ethanol extract and their concentrations by RP—HPLC

Name of compound	RT (min)	Standard Concentration	Standard peak area	Sample peak area	Ficus ethanol extract conc(mg/ml)
Ceffeic acid	28	100µgm	14950	3715.713	0.82
P-coumaric acid	33.2	100µgm	5043	1299.9	0.85
Rutin	37.02	100µgm	1928	982	1.68
Quercetin	45	100µgm	25340	1459	0 .19

Table7: Polyphenol compounds identified in Guava and their concentrations by RP—HPLC

Name of compound	RT (min)	Standard Concentration	Sample Concentration	Standard peak area	Sample peak area	Guava Methanol extract conc(mg/ml)
kaempferol	49.5	100µgm	30 µgm	5043	1299.9	0.85
Quercetin	45	100µgm	30 µgm	25340	1459	0.199
Rutin	37.02	100µgm	30 µgm	1928	982	1.68

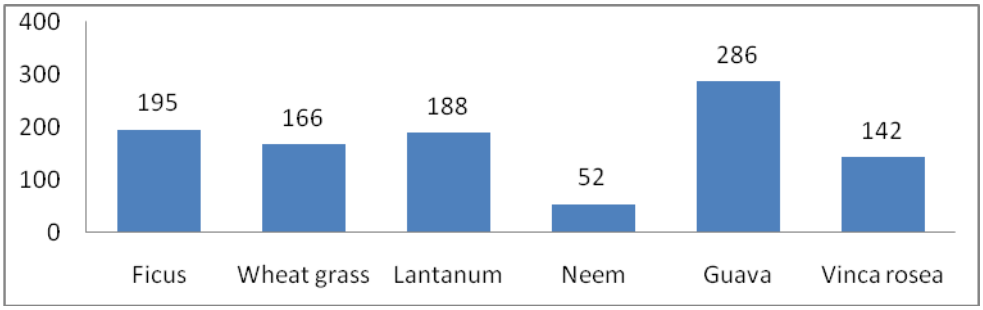

Fig.1 Total flavonoid content (µg/100µl) in different plant species

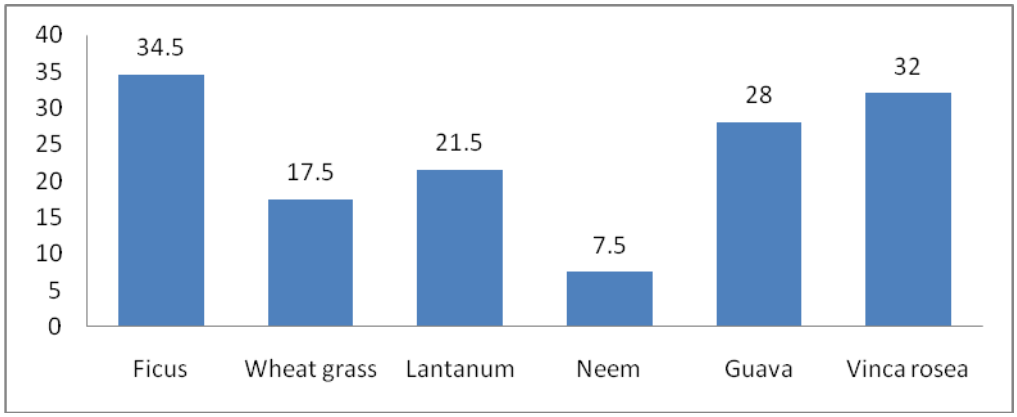

Fig.2 Total phenolic contents in different plant species

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Fig 3. Antibacterial Activity

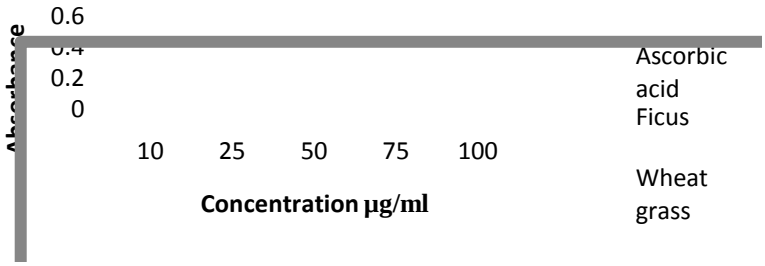

Fig:4 Ferric reducing power determination of ethanol extracts of various plants at different concentrations (µg/ml)

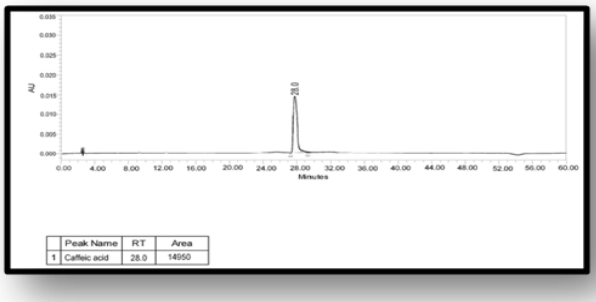

Fig. 5 Standard 1: Caffeic Acid

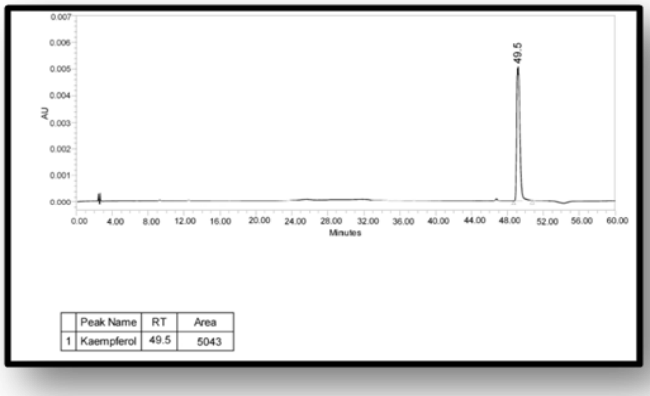

Fig. 6 Standard 2: Kaempferol:

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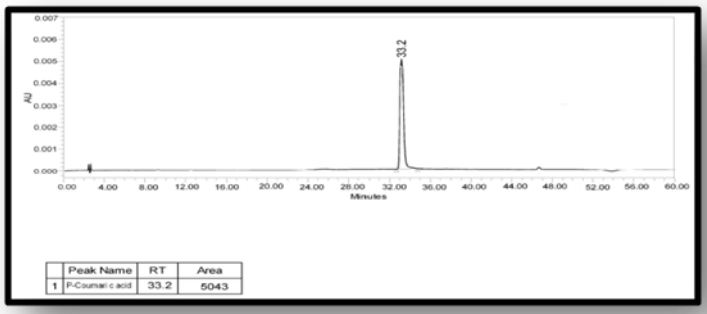

Fig. 7 Standard 3: P-Coumaric Acid

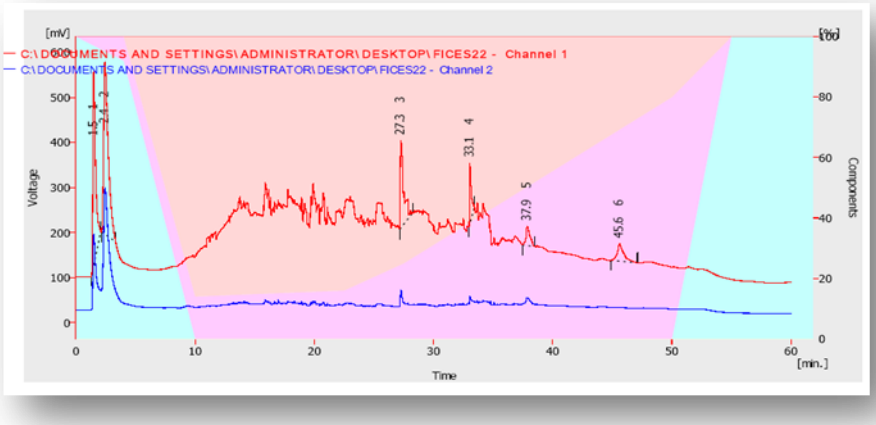

Fig:8 RP-HPLC chromatogram of methanol extract of Ficus religiosa

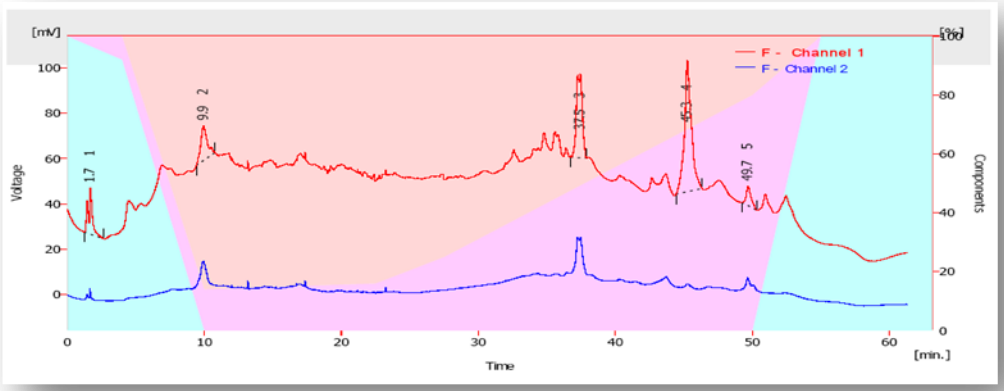

Fig.9 RP-HPLC chromatogram of methanol extract of Guava (*Psidium guajava*)