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EVALUATION OF PHYTOCHEMICALS AND *IN VITRO* PHARMACOLOGICAL ACTIVITY OF *ACACIA SINUATA* PLANT LEAVES

V. E. Vijayasekhar, M. Satya Prasad, D. S. D. Suman Joshi, K. Narendra, A. Krishna Satya and K. R. S. Sambasiva Rao

Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522510, India

Key words:

Acacia sinuata, Phytochemicals, antioxidant, antimicrobial activity.

Correspondence to Author:
K. R. S. Sambasiva Rao

Professor
Department of Biotechnology
Acharya Nagarjuna University
Nagarjuna Nagar, Guntur - 522510, A.P. India.

Email: krssrao@gmail.com

ABSTRACT: Objective: The present work is aimed to screen phytochemicals, in vitro antimicrobial and antioxidant activity of the non polar and polar extracts of *Acacia sinuata*. **Methods:** Leaves of *Acacia sinuata*- were collected, dried and powdered. The powdered material is subjected to soxhlet extraction using various polar to non polar solvents and allowed for evaporation. The crude extracts thus obtained were used for further screening of phytochemicals, *in vitro* antimicrobial activity and antioxidant activities. **Results:** Phytochemical evaluation of *Acacia sinuata* proved that most of the secondary metabolites are extracted in polar solvents than non polar solvent like hexane. All the extracts of the *Acacia sinuata* possess significant amount of phenols and flavonoids and they also showed antimicrobial and antioxidant activity. Out of all the extracts of *Acacia sinuata*, methanol extracts showed better antibacterial, antifungal activity and antioxidant activity. **Conclusion:** The present work concluded that the methanol extract of *Acacia sinuata* was useful to develop new plant based dugs against various pathogenic bacteria as well as fungal species.

INTRODUCTION: Many people believe that medicinal plants are more natural and more accessible than manufactured drugs as they were used in treating a wide spectrum of diseases [1]. According to Huang et al. (2008) [2], medicinal plants have been screened for their potential uses as alternative remedies. Plants are used in traditional medicine for their antibacterial, antifungal, and anticancer activities [3-4].

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Recently some products of higher plant origin have been shown to be effective sources of chemotherapeutic agents without undesirable side effects and strong biological activity [2]. Medicinal plants are considered as a source of biologically active biochemical like secondary metabolites, used for various applications in food, medicines, and industry [2].

India possesses an extremely rich biodiversity and these provide numerous plants with medicinal value. In a developing country like India Medicinal plants continue to be the main source of medication. From few decades, traditional system of medicine has become a topic of global importance. India is a unique country because of the presence of active stream of traditional system

of medicine which has indigenous Ayurveda. Many of the medicinal plants have been scientifically evaluated for their possible medical applications. The researchers can take a random approach to plant selection or can limit their search to plant of a certain species or genus (the taxonomic approach), plant that contain specific chemicals (the chemotaxonomic approach), or plant that are already known as traditional medical cures (the ethno botanical approach). Current developments in phytotechnology, photochemistry and biotechnology have facilitated rapid progress in natural product research. The present study describes the evaluation of phytochemicals and antimicrobial and antioxidant properties of *Acacia sinuata.*

Acacia sinuata is commonly known as Shikai, Shikaya, Chikaka, Shikakai, Banritha, Reetha, Kochi, Ritha, Sige. It is a climbing plant, most well-known for the natural shampoo derived from its fruit. Thorny branches have brown smooth stripes - thorns are short, broad-based, flattened. Leaves with caducous stipules are not thorn-like. Leaf stalks are 1-1.5 cm long with a prominent gland about the middle. Leaves are double-pinnate, with 5-7 pairs of pinnate, the primary rachis being thorny, velvety. Each pinna has 12-18 pairs of leaflets, which are oblong-lance shaped, 3-10 mm long, pointed, obliquely rounded at base. Inflorescence is a cluster of 2 or 3 stalked rounded flower-heads in axils of upper reduced leaves, appearing paniculate. Stalk carrying the cluster is 1-2.5 cm long, velvety. Flower heads about1cm in diameter when it matures. Flowers are pink, without or with reduced subtending bracts. Pods are thick, somewhat flattened, stalked, 8 cm long, 1.5-1.8 cm wide.

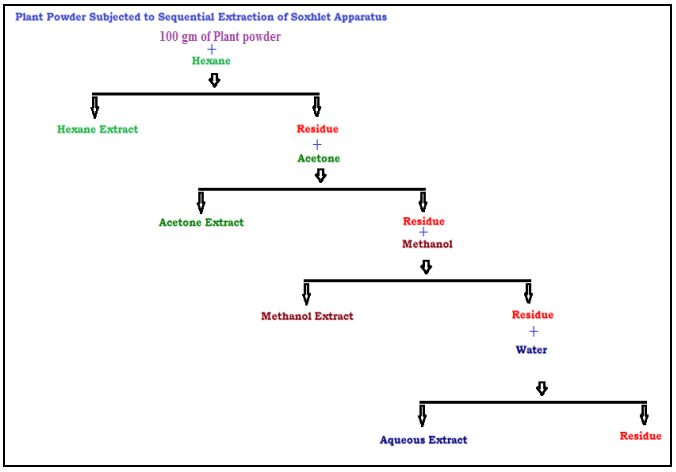
MATERIAL AND METHODS:

Collection of plant material:

The plant material was collected from the Seshachalam forest. The authentication was checked by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupathi, Andhra Pradesh. Required quantity of plant raw material i.e. leaves of *Acacia sinuata*, were collected and washed with running water followed by distilled water. Chopping process was carried out by separating the leaves from stems and they were allowed to dry under shade [5]. The dried material was stored in a sterilized polythene bags for further study.

Extraction technique:

The dried powder of the leaves was extracted sequentially [6] by soxhlet apparatus [7], using different solvents depending upon their polarities like Hexane, Acetone, Methanol and water (**Flow chart 1**). The extracts were concentrated and freed of solvent under reduced pressure, using rotary evaporator. The dried crude concentrated extracts were weighed to calculate the extractive yield and stored in air tight bottles, until used for analysis.



Phytochemical analysis:

Preliminary screening of Phytochemicals (Qualitative analysis):

Standard screening tests of four extracts *of Acacia sinuata*, were carried out to know the presence / absence of various secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures

Detection of Alkaloids:

Extract was dissolved individually in dilute Hydrochloric acid and the resultant solution was clarified by filtration.

Mayer's Test:

Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids.

Wagner's Test:
Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown / reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test:
Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test:
Filtrate was treated with Hager's reagent (saturated Picric Acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

Detection of Phenols:
Ferric Chloride Test: The filtered solution of extract was treated with three drops of freshly prepared 1% Ferric Chloride and Potassium Ferro cyanide. Formation of bluish- green colour is taken as positive.

Detection of Flavonoids:
Alkaline Reagent Test*:* The Extract was treated with few drops of Sodium Hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids.

Lead Acetate Test:
The Extract was treated with few drops of Lead Acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Anthraquinones:
Free Anthraquinones Test (Borntrager's test): The extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of Benzene, filtered and 5 ml of 10% Ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicates the presence of free anthraquinones.

Detection of Phytosterols:
Salkowski's Test: The extract was dissolved in 2 ml Chloroform in a test tube. Concentrated Sulfuric Acid was carefully added unto the wall of the test tube to form a lower layer. A reddish brown color at the interface indicates the presence of a steroid ring.

Detection of Terpenoids:
The extract was added to 2 ml of Acetic Anhydride and Concentrated H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids.

Detection of Tannins:
Ferric Chloride Test: The extract was dissolved in water and the resultant solution was clarified by filtration to which 10 % Ferric Chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black.

Lead Acetate Test:
The extract was dissolved in water and to that 10 % Lead Acetate solution was added. Appearance of yellow precipitate confirms presence of tannins.

Potassium Dichromate Test:
The extract was dissolved in water and to it a strong potassium dichromate solution was added. Yellow colour precipitate indicates presence of tannins and phenolic compounds.

Detection of Saponins:
Froth Test: Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

Detection of Anthocyanins:
The extract was added to 2 ml of 2 N HCl and Ammonia. Initial appearance of pink-red colour turning into blue-violet indicates the presence of anthocyanins.

Detection of Leucoanthocyanins:
The extract was added to 5 ml of Isoamyl Alcohol. Appearance of red upper layer colour indicates for presence of leucoanthocyanins.

Detection of Coumarins:
Three (3) ml of 10% NaOH was added to the extract. Formation of yellow colour indicates the presence of coumarins

Detection of Reducing Sugars:
Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Keller Kiliani test (for deoxy sugars in cardiac glycosides): Fifty **(**50) mg of each extract was dissolved in 2 ml chloroform. H_2SO_4 was added to form a layer and presence of colour at inter phase was noted. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

Quantitative analysis of Phenols and Flavonoids:
The quantity refers to the intrinsic value of the drug *i.e.*, the amount of medicinal principles present. The active constituents were glycosides, tannins, flavonoids, phenolic compounds, alkaloids, proteins and vitamins. The biological activity of a plant was influenced by the presence of various phytoconstituents. Natural antioxidants such as Vitamin C and Vitamin E directly influence the activity. Certain phyto constituents such as phenols, flavonoids, tannins, carbohydrates, proteins, Vitamin C and Vitamin E were known to act synergistically. Hence, they have to be quantified in the plant extract.

Determination of total phenol content:
The amount of total phenol content, in different solvent extracts of *Acacia sinuata* was determined by Folin- Ciocalteu's reagent method [8], 0.5 ml of extract and 0.1 ml (0.5 N) Folin- Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of extracted compounds).

Determination of total flavonoid content: The amount of flavonoid content in different solvent extracts of *Acacia sinuata* was determination by aluminium chloride colorimetric method [9]. The reaction mixture 3 ml consisted of 1 ml of sample (1 mg/ml) and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm. Rutin was used as positive control. The flavonoid content is expressed in terms of rutin equivalent (mg/g of extracted compound).

Antimicrobial Study:
Antimicrobial activity is expressed as zone of inhibition in millimeters, which is measured with a zone reader. The Hexane, Acetone, Methanol and Aqueous extracts of *Acacia sinuata* were screened for antimicrobial activity against a wide spectrum of microorganisms and the activity of extracts was compared with appropriate reference standards (Streptomycin for both gram positive and gram negative organisms and fluconazole for fungal strains). Microorganisms were grown in nutrient agar medium. Dimethyl Sulphoxide C and distilled water were used as control.

Test organisms:
The microorganisms used for the experiments were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh.

Gram-positive organisms:

Staphylococcus aureus(MTCC 3160)

Streptococcus mutans(MTCC 497)

Lactobacillus casei(MTCC 1423)

Lactobacillus acidophilus (MTCC495)

Bacillus megaterium(NCIM 2187)

Gram-negative organisms

Enterococcus faecalis(MTCC439)

Xanthomonascampestris(MTCC2286)

Escherichia coli(ATCC35218)

Pseudomonas aeruginosa (ATCC 9027)

Fungal strains:

Candida albicans(ATCC 10231)

Aspergillusniger(ATCC 1015)

Rhizopusoryzae(MTCC 262)

Candida rugosa(ATCC 96275)

Antimicrobial activity of selected medicinal plants: [10]

In vitro testing of the sensitivity bacterial and fungal isolates to antimicrobial agents using the disc diffusion assay, according to the guidelines set by the National Committee for Clinical Laboratories Standards (NCCLS, 1997). Antimicrobial activity was screened by agar well diffusion method [10]. The extracts were tested for antimicrobial activity against and gram positive, gram negative bacteria and fungi.

The Hexane, Acetone, Methanol and Aqueous extracts of *Acacia sinuata* were prepared separately at different concentrations such as 100µg/ml, 200µg/ml 300µg/ml, 400µg/ml and 500 µg/ml by using Dimethyl Sulphoxide as solvent (DMSO). Streptomycin (2µg/ml) and fluconazole (10µg/ml) were used as positive control (standard) for bacteria and fungi respectively. DMSO was used as negative control. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to 8 °C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of Dimethyl Sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

Anti oxidant Activity:

DPPH(2,2-diphenyl-l-picryl hydrazyl) Radical Scavenging Assay :

The antioxidant activity of the plant extracts was estimated using the DPPH radical Scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol and allowed overnight in the dark for generation of DPPH radical. A stock solution of Hexane, Acetone, Methanol, aqueous extracts of *Acacia sinuata*, and standard ascorbic acid were prepared in the concentration of100 mg/100ml (1mg/ml). From each stock solution 1ml, 2ml, 3ml, 4ml &5ml of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10 ml whose concentration was then100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml &500µg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 517 nm against the blank. For the control, 1 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min.

The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation [11-13].

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. IC50values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Ferric ion Reducing Antioxidant Power (FRAP assay):

Ferric ion reducing Antioxidant power was determined by the assay. The sample of the extract of 0.1ml was taken as test sample which was mixed with the 3.0 ml of working FRAP reagent then vortexing the solution. Sample was placed at 37^0C in water bath and then the absorbance was measured at 593nm. Ascorbic Acid standard (1000µM) were processed in the same way.

$$\text{FRAP scavenging activity (\%)} = \left[\frac{(A0 - As)}{A0}\right] * 100$$

Where, $\mathbf{A_0}$ is the absorbance of the control and as is the absorbance of the plant sample,

Concentration of working extract is 1mg/ml.

RESULTS AND DISCUSSION:

The physicochemical characteristics of the *Acacia sinuata*, extracts showed the preliminary information of such plant extracts. The successive extracts of plant material with non polar to polar solvents resultants were tabulated in **Table 1**. Variation in the colour of the extracts showed the variability of the presence compounds in the solvent extracts, and it also proved that variation in the dissolution of bioactive compounds from non polar to polar solvents. The tabulated results and graphical representation showed (**Fig.1**) that yield of extract has been increasing from non polar solvents to the polar solvents [14].

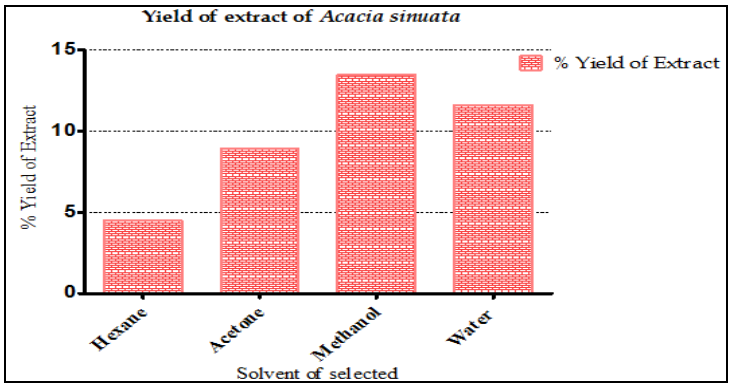


FIG.1: PHYSICOCHEMICAL CHARACTERISTICS OF *ACACIA SINUATA*

TABLE 1: PHYSICOCHEMICAL CHARACTERISTICS OF *ACACIA SINUATE*

Solvent	Initial Weight of the Powder (g)	Final Weight of the Powder (g)	Weight of the Crude Extract (g)	Crude Extract %	Colour of the Extract
Hexane	100	95.25	4.75	4.75	Dark Brown
Acetone	100	90.2	9.8	9.8	Dark Green
Methanol	100	86.1	13.9	13.9	Dark Green
Water	100	88.2	11.8	11.8	Dark Red

Preliminary screening:

The Preliminary qualitative analysis of the extracts showed the initial information of presence/ absence of the various metabolites in the plant extracts.

Qualitative analysis of*Acacia sinuata:*

The qualitative analysis of the *Acacia sinuata* extracts showed the phytochemical composition of the various extracts, and were tabulated in **Table 2.** The phytochemical analysis revealed that presence phenolics, steroids, saponins and reducing sugars in all the extracts of *Acacia sinuata*. Alkaloids, Flavanoids are found in all the above said extracts of *Acacia sinuata* except in hexane extract. Acetone and methanol extracts only showed the presence of tannins. Whereas anthroquinones, anthocyanins, leuco-anthocyanins and coumarins are completely absent in all the above said extractions.

TABLE 2: COMPARATIVE ANALYSIS OF PHYTOCHEMICAL ANALYSIS OF WHOLE ARIAL PART EXTRACTS OF *ACACIA SINUATA*

		Acacia sinuata			
S.No	Tests	Hexane Extract	Acetone Extract	Methanol extract	Water extract
1.			**Alkaloids**		
	Mayers	Negative	Positive	Positive	Positive
	Dragon	Negative	Positive	Negative	Positive
	Wagners	Negative	Positive	Positive	Positive
	Hagers	Negative	Negative	Negative	Negative
2.			**Phenolics**		
	$Fecl_2$ Test	Positive	Positive	Positive	Positive
3.			**Flavanoids**		
	Lead Acetate Test	Negative	Positive	Positive	Positive

	NaOH Test	Negative	Positive	Positive	Positive
4.			**Anthoquinone Test**		
	Borntrager's Test	Negative	Negative	Negative	Negative
5.			**PhytoSterols**		
	Salkowski's Test	Positive	Positive	Positive	Positive
6.			**Tannins**		
	$Fecl_2$ Test	Negative	Positive	Positive	Negative
	Lead acetate Test	Negative	Positive	Positive	Negative
	Pot. dichromate Test	Positive	Positive	Positive	Negative
7.			**Saponins**		
	Froth Test	Positive	Positive	Positive	Positive
8.			**Anthocyanins**		
	Ammonia-HCl Test	Negative	Negative	Negative	Negative
9.			**Leuco- Anthocyanin**		
	Iso Amyl Alcohol Test	Negative	Negative	Negative	Negative
10.			**Coumarins**		
	NaOH Test	Negative	Negative	Negative	Negative
11.			**Reducing Sugars**		
	Fehling's Test	Positive	Positive	Positive	Positive
	Keller-Kiliani Test	Positive	Positive	Positive	Positive

The phytochemical screening of various extracts of *Acacia sinuata*, proved that most of the secondary metabolites are extracted in polar solvents than non polar solvent like hexane. These results also said that the methanol extract possess more number of phytometabolites than other extracts.

Quantitative analysis of Phenols and Flavonoids

Total Phenol Content of *Acacia sinuate:* The total phenol content of the *Acacia sinuata* extracts were determined by folin ciocalteu method where Gallic acid was used as a standard control [15]. The quantitative analysis results of above said extracts were tabulated in **Table 3**. These results showed (**Fig.2**) that hexane extracts contain less amount of phenol when compared with polar solvent extracts, whereas methanol extract of above said plants contain high quantity of phenol followed by water and acetone extracts.

TABLE 3: TOTAL PHENOL CONTENT OF *ACACIA SINUATA*

concentration of extracts (µg/ml)	% of Phenol content µg GAE/µg			
	Hexane extract	Acetone extract	Methanol Extract	Water Extract
100	15.24	21.20	25.60	22.50
200	23.12	33.80	38.20	36.90
300	32.13	42.60	52.90	49.60
400	38.16	50.60	68.50	59.60
500	48.16	58.12	80.40	70.20

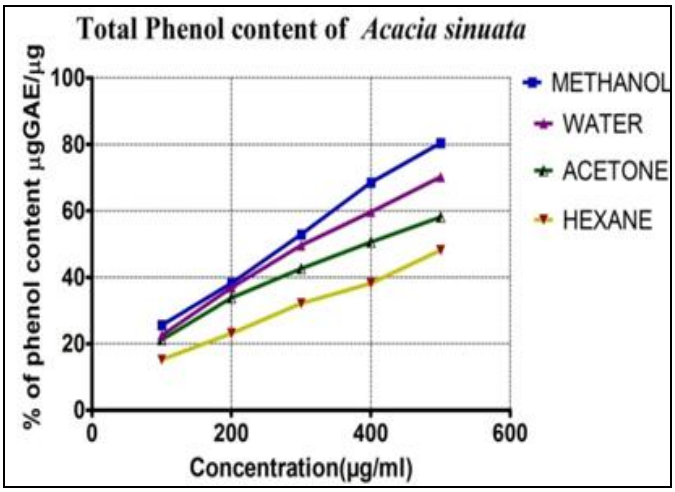


FIG.* 2: TOTAL PHENOL CONTENT OF *ACACIA SINUATA

Phenols are the effective natural antioxidant compounds that produce the reactive oxygen species with the free electrolytes present in the body. In the present study the amount of phenols present in the four extracts was estimated. The results showed that the phenols were higher in methanol extract as compared to other three extracts. Methanol extract showed dose dependent activity i.e. by increasing the concentration, the amount of phenols increased gradually. Aqueous extract contained significantly more phenols as compared to acetone and hexane extracts.

Total Flavonoid content of *Acacia sinuate*:

In the present study the quantification of flavanoids in the extracts of *Acacia sinuata, was* determined by aluminium chloride colorimetric method where rutin was used as a positive control. The results of all these plant extracts were tabulated in **Table 4**. The tabulated results and graphical representation showed (**Fig.3**) that the flavanoids were higher in methanol extract as compared to other three extracts. Methanol extract showed dose dependent activity i.e. by increasing the concentration, the amount of flavonoids increased gradually. Aqueous extract contained significantly more amount of flavonoids as compared to acetone and hexane extracts.

TABLE 4: TOTAL FLAVONOID CONTENT *ACACIA SINUATA*

% of Flavonoid content µg Rutin/µg				
concentration of extracts (µg/ml)	Hexane Extract	Acetone Extract	Methanol Extract	Water Extract
100	0.00	9.06	11.20	10.32
200	0.00	14.68	17.10	15.78
300	0.00	21.80	24.46	22.18
400	3.90	28.20	32.80	26.52
500	4.24	36.50	40.18	32.10

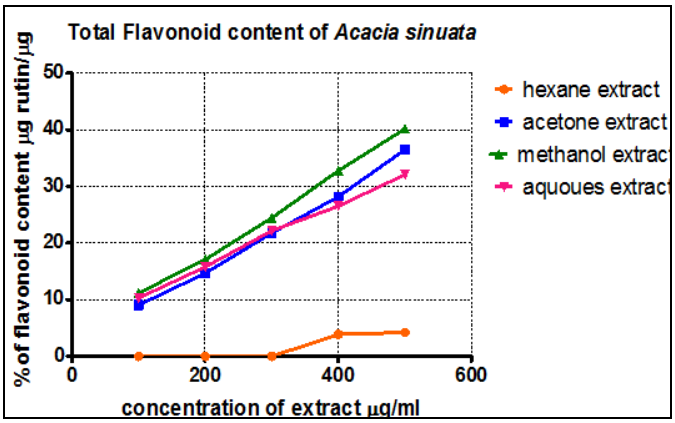


FIG. 3: TOTAL FLAVONOID CONTENT *ACACIA SINUATA*

The bioactivity of flavonoids depends on the number of hydroxyl groups, functional groups on the structure. Flavonoids have protective activities on humans which include coronary heart disease prevention, anti-inflammatory, antioxidant activity, hepato protective and anticancer activities. Flavonoids are being produced in bulk in the pharmaceutical industry with the aid of microbial biotechnology. Nonetheless, plants also contain useful and efficient quantities of flavonoids which can be used for betterment of human health[16].

Antimicrobial Activity:

Antibacterial Activity on gram +ve strains:

The antimicrobial activity of plant crude extracts depends on the dose and the type of bacterial strains employed. Also this antibacterial actions could be related to their chemical components in the crude extracts. The antimicrobial activity of four extracts of *Acacia sinuata* were tested against five gram positive bacteria-*Staphylococcus aureus, Streptococcus mutans, Lactobacillus casei, Lactobacillus acidophilus, and Bacillus megaterium.* After proper incubation the results were recorded and represented in **Fig.4.** Among the above extracts methanol extract of the *Acacia sinuata* showed a greater activity than the other extracts. These results also proved that *Bacillus megaterium* was more sensitive to all the plant extracts followed by *Lactobacillus acidophilus, Lactobacillus casei, Staphylococcus aureus and Streptococcus mutans.* Antimicrobial activity of the above plant extracts against gram positive organisms also proved that methanol is the most effective solvent for extracting broad spectrum of antimicrobial compounds from plant origin. Water and acetone extracts also showed the moderate antimicrobial activity, but hexane extract showed lesser activity.

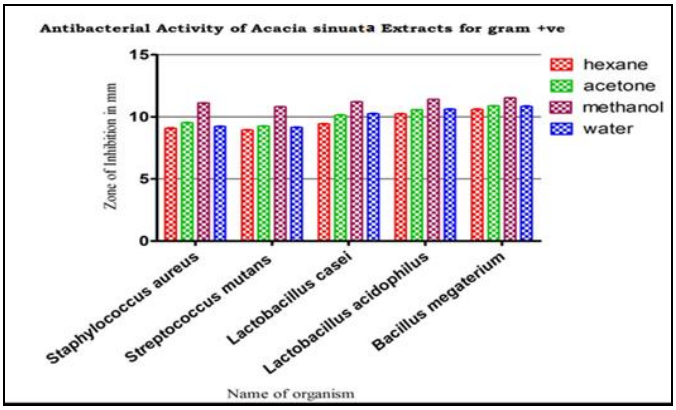


FIG 4: ANTIMICROBIAL ACTIVITY OF *ACACIA SINUATA* EXTRACTS FOR GRAM $^{+VE}$

Antibacterial Activity on gram -ve strains:

Most of the pathogenic bacteria belong to gram negative, they causes several diseases like sexually transmitted diseases, respiratory diseases, gastrointestinal problems, nosocomial infections etc. Owing to these problems, researchers show more interest to isolate potential drugs against gram negative organism from plant origin

In the present study, four gram negative pathogenic microorganisms were selected and tested against four extracts of *Acacia sinuata*. After proper incubation the diameter of inhibition zone was measured and results were recorded and represented in **Fig. 5**. Based on the comparative analysis, the methanol extract of *Acacia sinuata* showed better activity than the other extracts against above said gram negative organisms, followed by acetone. Water and hexane extracts.

These studies may helpful for the further isolation of antimicrobial drugs against gram negative organisms. The results of our research also highlight the fact that the methanol solvent extracts exhibited greater antimicrobial activity. So the present observation suggests that the methanol solvent extraction was suitable to verify the antimicrobial properties of medicinal plants which are also supported by many other investigators [17-20].

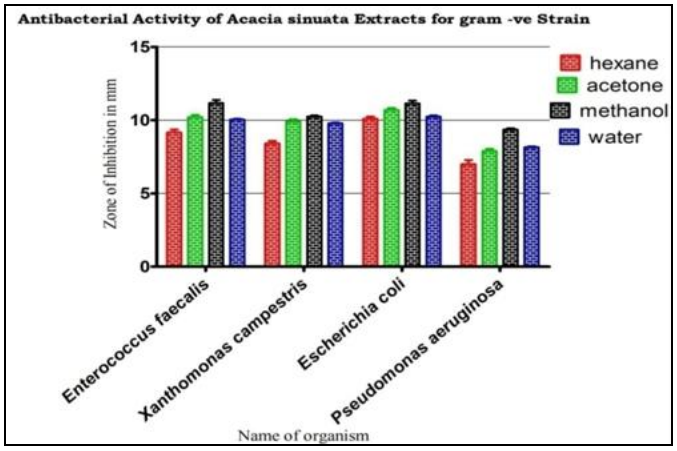


FIG. 5: ANTIMICROBIAL ACTIVITY OF *ACACIA SINUATA* EXTRACTS FOR GRAM -VE

Anti fungal Activity:

In the present study all the extracts of *Acacia sinuata*, showed antifungal activity and the results were recorded and represented in **Fig. 6**. From these results it was proved that Methanol extracts of above said plants showed highest antifungal activity, followed by water and acetone. Whereas hexane extract found lesser antifungal activity than other extracts. Out of three experimented plants, *Acacia sinuata* extracts found to possess highest antifungal activity against *Candia albicans, Aspergillus niger, Rhizopus oryza and Candida rogasa.*

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [21]. Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries [22]. In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. The antifungal activity of these plant extracts makes them potential source of antifungal agents and may be of economic importance as source of antifungal natural plant products.

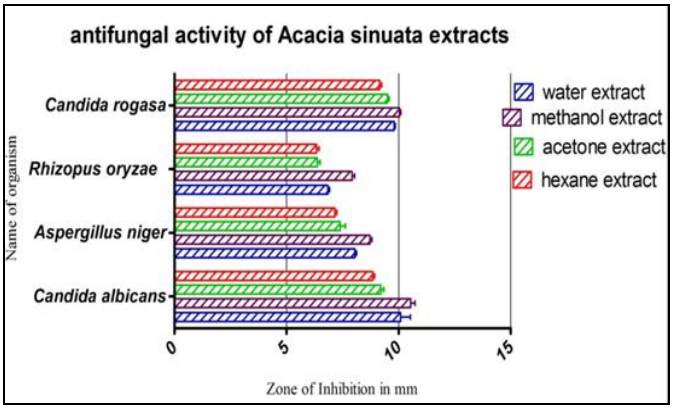


FIG. 6: ANTI-FUNGAL ACTIVITY OF *ACACIA SINUATA*

Anti oxidant activity:

The DPPH activity results of three plant extracts were tabulated and calculated the IC_{50} for these results. From the results (**Fig.7**), it was proved that the extracts of *Acacia sinuata*, possess hydrogen donating capabilities and it will act as an antioxidant. The results from this experiment revealed that the methanol extracts of screened plants showed highest antioxidant capacity followed by water and acetone extract. Whereas hexane extract showed lowest antioxidant activity. Out of all the extracts *Acacia sinuata* methanol extract showed the maximum antioxidant potentiality and lower IC_{50} value. These results indicated that the phenolic compounds (phenols and tannins) had a major contribution to the antioxidant activity of the plants. The parameter

IC_{50} (efficient concentration‖ value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). The methanol extracts of these plants showed low IC_{50} concentration (**Table 5)**.

TABLE 5*: DPPH RADICAL SCAVENGING ANTIOXIDANT ACTIVITY OF *ACACIA SINUATE*

Conc (µg/ml)	Hexane extract	IC 50	Acetone extract	IC 50	Methanol extract	IC 50	Water extract	IC 50	Standard % of inhibition (Ascorbic acid)	IC 50
100	28.2	448.2	29.5	396.95	37.8	269.85	34.5	365.93	50.67	107.02
200	33.4		35.7		46.3		40.2		53.25	
300	41.8		44.5		52.3		46.6		79.08	
400	47.4		50.6		58.8		51.3		83.51	
500	52.8		56.7		65.8		58.7		91.26	

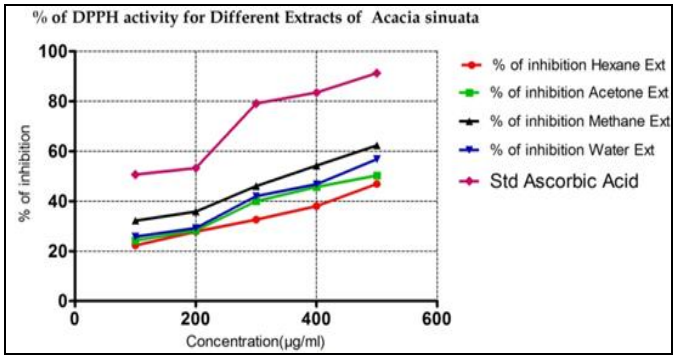


FIG.7: DPPH RADICAL SCAVENGING ANTIOXIDANT ACTIVITY OF *ACACIA SINUATA*

FRAP radical scavenging antioxidant activity:

The FRAP assay is a simple and inexpensive procedure that measures the total antioxidant levels in a sample. The method measures the reducing ability of anti oxidants against the oxidative effects of ROS. The higher the absorbance, the higher is the antioxidant activity which is indicated by the high FRAP value. In the reducing power assay, the extracts of *Acacia sinuata*, showed a concentration dependent antioxidant potential. In this assay, the presence of antioxidants in the extracts cause the reduction of the ferric cyanide complex in the ferrous form, leading to a color change of the test solution from yellow to different shades of green and blue, depending on the reducing power capacity of each tested extract. Therefore, Fe^{2+}concentration can be monitored by measuring the formation of Pearl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates an increase in reducing power. Among all the tested fractions, methanol extract of the *Acacia sinuata* exhibited highest FRAP Value followed by water, acetone and hexane extracts. The results of FRAP assay are depicted in **Table 6** and **Fig.8**.

TABLE6: FRAP RADICAL SCAVENGING ANTIOXIDANT ACTIVITY OF *ACACIA SINUATA*

Conc µg/ml	Hexane	IC_{50}	Acetone	IC_{50}	Methanol	IC_{50}	Aqueous	IC_{50}	Ascorbic Acid	IC_{50}
100	15.6	677.25	16.90	605.88	21.8	387.93	17.8	512.00	45.00	160.50
200	18.9		21.40		25.4		22.4		50.90	
300	26.6		28.60		38.5		30.8		66.40	
400	32.0		36.40		46.7		41.8		75.80	
500	40.1		43.80		54.8		49.8		83.00	

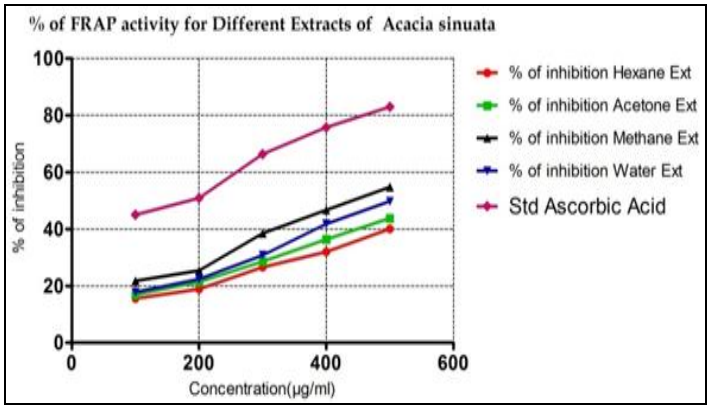


FIG.8: FRAP RADICAL SCAVENGING ANTIOXIDANT ACTIVITY OF *ACACIA SINUATA*

CONCLUSION: The current research work on phytochemical evaluation of *Acacia sinuata* proved that most of the secondary metabolites are extracted in polar solvents than non polar solvent like hexane. All the extracts of the *Acacia sinuata* possess significant amount of phenols and flavonoids and they also showed antimicrobial and antioxidant activity. Out of all the extracts of *Acacia sinuata*, methanol extracts showed better antibacterial, antifungal activity and antioxidant activity. The present work concluded that the methanol extract of *Acacia sinuata* was useful for the discovery of new plant based dugs against various pathogenic bacteria as well as fungal species.

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