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PHYTOCHEMICAL SCREENING, ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF MEDICINAL PLANTS

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ABSTRACT

Many Indian plants have been investigated for their beneficial use in different diseases and reports occur in numerous scientific journals. This work was mainly concerned with the identification of the therapeutic properties of *Adhatoda vasica*, *Biophytum sensitivum* and *Caesalpinia bonduc* Extracts. The leaf extracts of *Adhatoda vasica*, *Biophytum sensitivum* and *Caesalpinia bonduc* was used for its anti-oxidant and antimicrobial activity. Among the extracts, Ethanolic extract showed highest anti-oxidant property. The ethanolic extract was further studied and phytochemicals were identified. The ethanolic extract of *Adhatoda vasica*, showed highest anti-microbial activity against pathogenic bacteria such as *E.coli* and *Staphylococcus aureus* when compared to other extracts.

KEYWORDS: Antioxidant activity, Anti-microbial activity, phytochemical screening, *Adhatoda vasica*.

INTRODUCTION

Ayurveda literally means science of life which originated from prehistoric antiquity, but its concepts matured between 2500 BC and 500 BC in India. It has a vast literature in Sanskrit and various Indian languages, conversing all aspects of diseases, therapeutics and pharmacy. Plants form a dominant part of ayurvedic pharmacopoeia along with animal Products, mineral and metals. Vegetable products dominated Indian Materia Medica which made extensive use of bark, leaves, flowers, fruits, roots, tubers and juices. Rauwolfia and guggul (Commiphora wightii) are indicative of the untapped wealth of the medicinal plants of Ayurveda. Sukh Dev

(Sukhoev et al, 1997) listed 15 crude Ayurvedic drugs which have received support for their therapeutic claims.

Mankind has been using plant materials for treatment of his ailments since time immemorial. Since ancient times, before modern medicine, people became ill and suffered from various ailments. In the absence of modern medicinal remedies people relied on herbal remedies derived from plants and herbs. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro et al, 2000). Medicinal plants, herbs, spices and herbal remedies are known to Ayurveda in India since long times. History of herbal remedies is very old. The ayurvedic drugs have stood the test of time for their safety, efficacy, cultural acceptability and toxicity. About 75-80% of the world population, mainly in the developing countries still use plant based medicines for primary health care. Plants have also been a significant source of the modern day drugs. Many of the currently available drugs were derived directly or indirectly from phytochemicals (Grover et al, 2002).

There are less than 1% of some 250,000 higher plants that have been screened in-depth for their phytochemistry or pharmacology (Petlevski et al, 2001). However, herbs are staging a comeback and 'herbal renaissance' is happening all over the globe. Herbal medicines are still being used in many countries, because of their compatibility with the human body, cheapness, and minimum side effects. During the last decade there has been a major increase in the use of medicinal plants all over the world particularly in U.S.A. and European countries. The World Health Organization estimated that about 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, which primarily involves the use of plant extracts or their active components. The beneficial medical effects of plant materials typically result from the combination of secondary products present in the plant; majority of these are secondary metabolites like alkaloids, tannins and phenolic compounds. These secondary metabolites exert their effects by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or even neurotransmitters. Hence the screening of plants for such properties is an important step. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs have been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpasses their importance, for a while. However, the blind dependence on synthetics is over and people are returning to

the naturals with hope of safety and security. Over three-quarters of the world population nowadays relies mainly on plants and plant extracts for health care (Fabricant et al, 2001).

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

The leaves of *Adhatoda vasica, Biophytum sensitivum* and *Caesalpinia bonduc* were obtained from medicinal plant vendor in Hyderabad, Telangana, India.

AUTHENTICATION OF PLANTS

The selected plant materials were identified and authenticated as ADVA/2016/243 for *Adhatoda vasica*, *BISE*/2016/165 and as CABO/2016/418 for *Caesalpinia bonduc* by Botanist Prof. C. Prabhakar Raju, S.S.B.N Degree and P.G. College, Sri Krishnadevaraya University, Andhra Pradesh, India. The leaves were shade dried at room temperature for a week and made into coarse powder.

EXTRACTION OF PLANTS

To prepare ethanolic extract of leaf powder the plant material (1 kg) was soaked in ethanol (3 volumes) in a glass jar for 2 days at room temperature and the solvent was filtered. This was repeated 3 to 4 times until the filtrate gave no coloration. The filtrate was concentrated under reduced pressure in the Buchirota vapour R-200 and finally freeze dried, which was used in bioassays.

INVITRO EFFICACY EVALUATION

Dried plant extracts were dissolved in the respective solvents at the stoc 0.8 ml of concentration of 1 mg/ml. The appropriate dilutions of the stock solutions were made and used for the invitro antioxidant assays.

Free radical scavenging activity was determined by the method of Koleva, et al., (2002).

DPPH radical scavenging activity

To 0.1 ml of ethanolic solution of DPPH an equal volume of test compound was added at different concentrations in methanol. Equal volume of methanol was added to control. Above mixture was kept at room temperature for 20 minutes for incubation. Absorbance was recorded at 517 nm. Scavenging capacity was calculated by monitoring the decrease in absorbance at 517 nm. The antioxidant activity of test drug was expressed as IC_{50}.

Percentage Inhibition was measured by using formula;

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically by the method of Govindarajan, et al., (2003).

Nitric oxide from sodium nitroprusside in aqueous solution at pH interacts with oxygen to generate nitrite ions, which were measured by the Griess reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10mM) in phosphate-buffered saline and various concentrations of Medicinal plants Aqueous, hydroalcoholic and methanolic extracts at different concentrations were incubated at 25ºC for 150 min. A 0.5-ml aliquot of the incubated sample was removed and 0.5 ml Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% phosphoric acid) was added. The absorbance of purple chromophore formed during diazotization of nitrite along with suphanilamide and subsequent coupling with napthyl ethelene diamine was measured at 546 nm.

Percent inhibition was measured by comparing the absorbance values of test samples as per the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Antimicrobial Activity (Amjad Khalil et al 2012)

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 3160), *Bacillus subtilis* (MTCC 441), *E.Coli* (MTCC 443) and *Klebsiella pneumonia* (MTCC 13883), fungal strain *C.albicans* (MTCC 227) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antimicrobial activity. The fungal and bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium following refrigeration storage at 4°C. The bacteria were grown on Mueller-Hinton agar plates at 37°C, whereas the fungi were grown in dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Determination of zone of inhibition method

Preparation of Discs

Whatman No.1 filter paper discs of 5mm diameter were autoclaved by keeping in a clean and dry Petri plate. The discs were soaked in plant extracts for 5 hours were taken as test material. After 5 hours the discs were shade dried. The concentrations of plant extracts per disc are accounted for 0.1 grams/1ml. Subsequently they were carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, methanolic, benzene and ethyl acetate are prepared and used as control.

Testing of antimicrobial activity

To test the antimicrobial activity, LB agar medium was prepared and the medium was sterilized at 121°C for 30 min's. The agar plates were prepared by pouring about 10 ml of the medium into 10 cm Petri dishes under aseptic condition and left undisturbed for 2 hrs to solidify the medium. 1 ml of inoculum (containing suspension) of bacteria was poured on to the plates separately containing solidified agar media. The prepared sterile filter paper discs were impregnated with the extracts and shaken thoroughly and this test plates incubated for a period of 48 hrs in BOD at 37ºC for the development of inhibitory zones and the average of two independent readings for each organism in different extracts were recorded.

Measuring the diameter of inhibition zone

The inhibition zones were measured after 1 day at 37ºC for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. Five paper discs placed in one Petri plate.

PHYTOCHEMICAL ANALYSIS

Chemical tests were carried out on the ethanolic extracts using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1984).

Detection for flavonoids (HARBORNE, 1984)

Shinoda test: A small piece of magnesium ribbon was added to the alcoholic solution of the extract followed by drop wise addition of concentrated hydrochloric acid. The green blue color indicates the test is positive.

Detection for alkaloids (Sofowara 1993)

A small portion of solvent free extract was stirred with few drops of dilute hydroalcoholic acid and filter. The filtrate was tested with following reagents.

a. Dragendrof reagent (Potassium bismuth iodide) - To 2-3 ml filtrate, few drops of the reagent was added. Orange brown precipitate is formed.

b. Mayer's reagent (Mercury potassium iodide) – To 2-3 ml filtrate, few drops of the reagent added gives cream precipitate.

c. Hager's reagent (Saturated picric acid)- With 2-3 ml of filtrate the reagent gives yellow precipitate.

d. Wagner's reagent (Iodine reagent)-With 2-3 ml of filtrate the reagent gives reddish brown precipitate.

Detection of Triterpenoids (Trease and Evans 1989)

a. Libermann-Burchard test - Chloroform solution of hydrolysate was treated with acetic anhydride and sulphuric acid. Formation of blue or blue–green colour indicated the presence of steroidal saponins whereas red, pink or violet colour indicated the presence of triterpenoids saponins.

Detection of Saponin and Glycosides (Sofowara 1993)

A small portion of the extract was hydrolyzed by boiling with dilute hydrochloric acid for few min and hydrolysate was subjected to following tests.

b. Legal's test - The hydrolysate was dissolved in pyridine and solution of sodium nitroprusside was added to it and made alkaline. Formation of pink or red colour indicated the presence of cardiac glycosides.

c. Borntrager's test - An organic solvent like ether or chloroform was added to the hydrolysate and the contents were shaken. The organic layer was shaken and treated with solution of ammonia. The development of pink colour indicated the presence of anthraquinone glycosides.

9. Test for Saponin Glycosides

Foam test: About 1 ml of extract was diluted with water to 20 ml and shaken in a graduated cylinder for 15 min. A 1 cm layer of foam indicates presence of saponins.

Detection of phenolic compounds and tannins (Harborne 1984)

A small quantity of extract was diluted with water and tested with following reagent.

a) Dilute ferric chloride (FeCl3) solution (5%) Intense blue, green, red or purple color indicates the presence of phenolic compounds. An appearance of violet color indicates the presence of tannins.

b) Acetic acid solution: Forms red color solution indicating presence of phenolic compounds

c) Solution of gelatin (1%) containing 10% sodium chloride (NaCl)- Precipitate indicates positive test for tannins.

d) Lead acetate solution (10%)-Gives buff colored precipitate for phenolic compounds.

RESULTS AND DISCUSSION

The leaves of *Adhatoda vasica, Biophytum sensitivum* and *Caesalpinia bonduc* forms fresh plants were used for the study. Different fractions for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activities in Table 1. Ethanolic extract showed the highest scavenging activity followed by Ethyl acetate. DPPH scavenging activity was significantly correlated with phenolics and flavonoids in different extracts.

Based on the experimental results, among all the extracts, ethanolic extract showed higher scavenging activity towards DPPH. These extracts have shown a strong inhibitory effect on DPPH radical at 250 µM concentration and inhibition rates were: 96.18% ± 1.42% (ethanolic1), 96.50% ± 1.39% (for methanolic1), 97.26% ± 1.12% (for ethanolic2) and 98.39 ± 1.12 better than the positive control BHT (76.45 ± 1.42%) (Table 1, 2 and 3).

Table 1: Antioxidant activity of *Adhatoda vasica* extracts by DPPH method.

Extracts					**Scavenging Effect (%)**	
-	**25 µM**	**50 µM**	**75 µM**	**100 µM**	**125 µM**	**250 µM**
Ethanolic1	40.54 ± 1.32	52.37 ± 1.35	64.86 ± 1.40	88.39 ± 1.45	95.19 ± 1.50	96.18 ± 1.42
Methanolic1	50.39 ± 1.18	53.58 ± 1.62	64.12 ± 1.34	86.69 ± 1.83	95.26 ± 1.87	96.50 ± 1.39
Ethylacetate1	19.14 ± 1.53	57.28 ± 1.23	61.23 ± 1.32	85.23 ± 1.42	94.45 ± 1.18	97.51 ± 1.12
Benzene1	25.88 ± 1.03	26.74 ± 1.32	43.30 ± 1.67	39.93 ± 1.49	45.24 ± 1.45	69.70 ± 1.68
Control	18.56 ± 0.95	28.36 ± 1.19	42.18 ± 1.48	45.58 ± 1.41	47.58 ± 1.54	76.45 ± 1.42

Table 2: Antioxidant activity of *Biophytum sensitivum* extracts by DPPH method.

Extracts	**Scavenging Effect (%)**					
-	**25 µM**	**50 µM**	**75 µM**	**100 µM**	**125 µM**	**250 µM**
Ethanolic2	42.44 ± 1.32	55.24 ± 1.35	66.56 ± 1.40	89.79 ± 1.45	96.38 ± 1.50	97.26 ± 1.42
Methanolic2	53.69 ± 1.18	56.27 ± 1.62	68.72 ± 1.34	88.39 ± 1.83	95.83 ± 1.87	97.31± 1.39
Ethylacetate2	40.54 ± 1.32	52.37 ± 1.35	64.86 ± 1.40	88.39 ± 1.45	95.19 ± 1.50	96.18 ± 1.42
Benzene2	27.68 ± 1.03	28.92 ± 1.32	45.40 ± 1.67	40.63 ± 1.49	48.18 ± 1.45	69.42 ± 1.68
Control	18.56 ± 0.95	28.36 ± 1.19	42.18 ± 1.48	45.58 ± 1.41	47.58 ± 1.54	76.45 ± 1.42

Table 3: Antioxidant activity of *Caesalpinia bonduc* extracts by DPPH method.

Extracts	Scavenging Effect (%)					
-	25 µM	50 µM	75 µM	100 µM	125 µM	250 µM
Ethanolic3	19.50 ± 1.53	59.36 ± 1.23	64.33 ± 1.32	87.43 ± 1.42	95.29 ± 1.18	98.39 ± 1.12
Methanolic3	27.68 ± 1.03	28.92 ± 1.32	45.40 ± 1.67	40.63 ± 1.49	48.18 ± 1.45	69.42 ± 1.68
Ethylacetate3	19.14 ± 1.53	57.28 ± 1.23	61.23 ± 1.32	85.23 ± 1.42	94.45 ± 1.18	97.51 ± 1.12
Benzene3	42.44 ± 1.32	55.24 ± 1.35	66.56 ± 1.40	89.79 ± 1.45	96.38 ± 1.50	97.26 ± 1.42
Control	18.56 ± 0.95	28.36 ± 1.19	42.18 ± 1.48	45.58 ± 1.41	47.58 ± 1.54	76.45 ± 1.42

Antibacterial Activity

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 3160), Bacillus cereus (MTCC 1305) *E.Coli* (MTCC 443) and *Pseudomonas aureo ginosa* (MTCC 2453) and *Candida albicans* were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antibacterial activity. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar. The bacteria were grown on Mueller-Hinton agar plates at 37°C. The stock cultures were maintained at 4°C for the growth of fungi potato dextrose agar was used.

Determination of zone of inhibition method

Preparation of Discs

Whatman No.1 filter paper discs of 5mm diameter were autoclaved by keeping in a clean and dry Petri plate. The discs were soaked in compound solutions for 5 hours were taken as test material. After 5 hours the discs were shade dried. The concentrations of compound solutions per disc are accounted for 0.1 grams/1ml. Subsequently they were carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, Hexane, benzene and distilled water are prepared and used as control.

Testing of antibacterial activity

To test the antibacterial activity, LB agar medium was prepared and the medium was sterilized at 121°c for 30 mins. The agar plates were prepared by pouring about 10ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inoculum (containing suspension) of *Staphylococcus aureus*, *Bacillus subtilis*, *E.Coli* and *Klebsiella pneumoniae* was poured on to the plates separately containing solidified agar media. The prepared sterile filter paper discs were impregnated with the compound solutions and shaken thoroughly and these test plates incubated for a

period of 48 hrs in BOD at 37ºc for the development of inhibitory zones and the average of 2 independent readings for each organism in different compound solutions were recorded.

Measuring the diameter of inhibition zone

The inhibition zones were measured after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. Five paper discs placed in one Petri plate (Table 4).

Table 4: Anti-bacterial activity of different extracts of medicinal plants.

Compound	Gram (–) veE.coli			Gram (+) ve Bacillus			Gram (+) Staphylococcus aureus			Gram (–) ve P.aureo ginosa			C.albicans (fungi)		
	200 µg	300 µg	500 µg	200 µg	300 µg	500 µg	200 µg	300 µg	500 µg	200 µg	300 µg	500 µg	200 µg	300 µg	500 µg
Ethanolic1	2 mm	2.5 mm	3 mm	5mm	6 mm	8mm	4mm	5 mm	6mm	2 mm	2 mm	3 mm	10mm	12mm	14 mm
Methanolic1	1 mm	1.5mm	2mm	6mm	6mm	8 mm	3mm	3mm	5mm	4mm	6mm	8 mm	8mm	9mm	9 mm
Ethylacetate1	2mm	2 mm	3mm	4mm	5mm	6 mm	5 mm	5mm	7mm	6 mm	7mm	8 mm	6mm	8 mm	10 mm
Benzene1	2mm	2.2 mm	3 mm	5mm	6mm	11 mm	4 mm	5 mm	6mm	8 mm	8 mm	9 mm	6mm	7mm	8 mm
Ethanolic2	4 mm	5 mm	6mm	6mm	8 mm	9 mm	8mm	9mm	10 mm	7 mm	8 mm	8 mm	5mm	6 mm	8 mm
Methanolic2	3mm	4 mm	5mm	5 mm	6mm	7 mm	4 mm	5 mm	8 mm	6mm	8 mm	9 mm	4 mm	6 mm	8 mm
Ethylacetate2	5 mm	6 mm	7 mm	4mm	5.5 mm	6.5 mm	9mm	11mm	12 mm	5mm	6 mm	6 mm	6 mm	7mm	8 mm
Benzene2	3 mm	4 mm	5 mm	3mm	4 mm	6mm	5mm	7 mm	9 mm	4mm	5 mm	6 mm	5mm	6mm	7mm
Ethanolic3	1 mm	1.5mm	2mm	6mm	6mm	8 mm	3mm	3mm	5mm	4mm	6mm	8 mm	8mm	9mm	9 mm
Methanolic3	4 mm	5 mm	6mm	6mm	8 mm	9 mm	8mm	9mm	10 mm	7 mm	8 mm	8 mm	5mm	6 mm	8 mm
Ethylacetate3	3mm	4 mm	5mm	5 mm	6mm	7 mm	4 mm	5 mm	8 mm	6mm	8 mm	9 mm	4 mm	6 mm	8 mm
Benzene3	2mm	2.2 mm	3 mm	5mm	6mm	11 mm	4 mm	5 mm	6mm	8 mm	8 mm	9 mm	6mm	7mm	8 mm
Streptomycin	5mm	6mm	7mm	6.6mm	7.5mm	9.5mm	10mm	12mm	13mm	6mm	8mm	10mm	6mm	8mm	10mm

In disc diffusion method the nutrient agar was poured into Petri plate and allowed to solidify. After 1ml of microbial suspension was poured into plates, series extracts were applied on to the discs and placed in Petri plate and incubated for 48 hours. The results from the agar plates revealed all compounds showed inhibitory zones. The 200µg, 300 µg, and 500 µg concentrations of ethanolic extract of *Adhatoda vasica* showed best inhibitory zones. The inhibitory zones represent the sensitiveness of microorganisms in culture to the compounds. In this study, *Staphylococcus aureus* was found to the most sensitive among the tested microorganisms.

Phytochemical Screening

By comparing all the zones of inhibition values we can say that *Staphylococcus aureus* and *E.coli* was sensitive even in low concentration. These inhibit *Staphylococcus aureus* and *E.coli* effectively at all concentrations.

Table 5-8 shows the presence of various phytochemicals in the different extracts of *Adhatoda vasica, Biophytum sensitivum* and *Caesalpinia bonduc*. It is observed from these tables that the ethanolic extracts of *Adhatoda vasica, Biophytum sensitivum* and *Caesalpinia bonduc* contained higher concentration of phenols. The presence of carbohydrates, flavonoids, tannins, terpenoids, alkaloids, steroids and phlobatannins were noted in both the extracts. However, the presence of saponins was noted only in *Caesalpinia bonduc* extract. Triterpenes were not seen all the Hexane extracts. All the plant extracts were devoid of coumarin, furanoid and amino acids.

Table 5: Phytochemical studies of *Adhatoda vasica.*

S.No	Secondary metabolites	Ethanolic
1	Steroids	+
2	Triterpenes	+
3	Saponins	-
4	Tri terpinoidal saponins	-
5	Alkaloids	+
6	Carbohydrates	+
7	Flavonoids	+
8	Tannins	+
9	Glycosides	+
10	**Polyphenols**	++

Preliminary phytochemical screening is important and useful for isolation of pharmacologically active compounds present in the plants, (Sugumaran & Vetrichelvan,

2008). This serves as an important tool for the quality assurance of plant for future studies. Till now almost all investigated plants showed to contain different active constituents of pharmacological importance in the form of secondary metabolites (Ming *et al*., 2005).

Qualitative phytochemical screening of the leaves, revealed that Proteins, carbohydrates and tannins were present in both aqueous and ethanolic extracts of all the parts. Alkaloids were detected in both aqueous and ethanolic extracts of leaf and stem. Flavonoids were present in both type of extracts of all parts except root. Saponins and glycosides were present in all extracts; however Glycosides were not detected in Borntrager test. Phytosterol and tri-terphenoids were present in all the parts, while spot test for fixed oil gave negative result. Volatile oil was only detected in the ethanolic and aqueous extracts. The result showed that these plants rich in bioactive compounds and hence is a potential source of therapeutic properties.

Khan *et al*., (2010) reported that the ethanolic extracts of the leaf, stem and root of *Pluchealanceolata* have almost the same phytochemical constituents as present in the present research plants. Arjun*et al*., (2009) also explored phytochemical composition of leaf of *Hygrophilaspinosa*. Chirikova *et al*., (2010) investigated preliminary phytochemical screening of *Scutellariabaicalensis*. Kalyan *et al.*, (2011) subjected ethanol extract of *Clitoriaternatea* seeds to preliminary phytochemical investigations. All these works emphasize on the importance of phytochemical screening for obtaining first hand information about metabolites of pharmacological significance (Table 6-8).

Table 6: Phytochemical studies of *Biophytum sensitivum*.

S.No	Secondary metabolites	Ethanolic
1	Steroids	+
2	Triterpenes	+
3	Saponins	-
4	Tri terpinoidal saponins	+
5	Alkaloids	+
6	Carbohydrates	+
7	Flavonoids	+
8	Tannins	+
9	Glycosides	+
10	**Polyphenols**	+

Table 7: Phytochemical studiesof *Caesalpinia bonduc.*

S.No	Secondary metabolites	Ethanolic
1	Steroids	+
2	Triterpenes	+
3	Saponins	+
4	Tri terpinoidal saponins	+
5	Alkaloids	+
6	Carbohydrates	+
7	Flavonoids	+
8	Tannins	+
9	Glycosides	+
10	**Polyphenols**	+

CONCLUSION

Phytochemicals present in the ethanolic extracts of *Adhatoda vasica*, *Biophytum sensitivum* and *Caesalpinia bonduc* were identified. Among the extracts ethanolic extract has highest anti-oxidant property when compared to other extracts. In the present study it was found that *Adhatoda vasica*, *Biophytum sensitivum* and *Caesalpinia bonduc* ethanolic extracts have an excellent antimicrobial activity. The pathogenic bacteria were inhibited in presence of the leaf extract of *Adhatoda vasica* more when compared to other plants. Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria.

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