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Preliminary phytochemical analysis and extraction of crude drugs from medicinal plants and their antimicrobial activity

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Abstract

Herbal treatments are becoming increasingly popular, as the herbal preparations have no or least side effects than synthetic drugs. In our present study, the various parts of medicinal plants (*Abutilon indicum, Balanites egyptiaca, Caesalpini bonduc*) are used in Ayurvedic and other folk medicines for the treatment of various diseases. This study was designed to evaluate the phytochemical and antimicrobial activities of medicinal plants extracted by the soxhlet extraction method with various solvents. The extracts of these various plants shows the antimicrobial activity against various micro-organisms and have effective for the treatments of alimentary canal, bronchitis, diarrhoea, inflammation , cough, skin, insect bite, abdominal pain, gastro-intestinal complaints and for proper regulation of menstrual flow etc.

Keywords: *Abutilon indicum, Balanites egyptiaca , Caesalpinia bonduc*, Phytochemical, antimicrobial, medicinal plants.

INTRODUCTION

Despite of the use of every existing ways of plant protection, about 33 percent of annual harvested food commodities of the globe is damaged by the pests and the loss which is approximated as 6000 corers annually. Speedy and efficient execution of plant diseases in agriculture commodities is commonly accomplished by using the artificial pesticides and industrialization.

However, unsystematic use of the artificial pesticides and modern technology has developed health hazards in humans as well as animals by means of residual toxicity. Pathovars of Xanthomonas are notorious to disease development on numerous vegetables, cash crops and are stated to have improved resistance to Ampicillin, Kanamycin, Streptomycin and Pencillin. The other disease developing microbes in humans and animals are like *E.coli*, *Candida albicans* (form of yeast), *Staphylococcus aureus* (facultative anaerobic gram-positive cocci), *Pseudomonas aeruginosa* (gram-negative bacterium). These microbes lead to many diseases for which the therapy is highly costly and those cannot be afforded by the below poverty line members, middle class peoples as well as tribals. The alternate for this is the use of medicinal plants which is the old technique coming from past centuries.

Medicinal plants are the ones thought to possess the medicinal properties, but some plants or the phytochemical ingredients of them have been proven by precise science and also got the approval by famous regulatory agencies like USFDA or EFSA to have medicinal effects. Plants have developed the capability to produce chemical compounds that actually aid them to defend themselves against insects, fungi and herbivorous mammal attacks. By chance, few of the compounds, whilst being toxic to plant predators, turn out to have favorable effects in human disease therapy. Such secondary metabolites are extremely diverse in structure; a lot of them are aromatic substances like phenols or oxygenated phenol derivatives.

Besides the production of plant primary metabolites, plants can produce secondary metabolites like flavonoids, alkaloids, reducing sugars, saponins, tannins, terpenoids etc., which are not frankly involved in organism development, growth and reproduction. The tribal practioners make use of these secondary metabolites in disease curing.

Unlike the primary metabolites the secondary metabolite absence does not leads to instantaneous fatality. However, there may be an impairment of organism survivability and fecundity in long term. They are often constrained to a narrow set of species with in a poly-genetic group and often play a vital role in plant defense against other species and herbivores. They are also useful to human beings as recreational drugs, medicines and flavorings.

Most of the secondary metabolites of interest to human kind fit into categories which classify secondary metabolites based on their bio-synthetic origin. Various classes of secondary metabolites include the **Alkaloids** the small and heavily derivatized amino acids like Atropine of *Atropa belladonna*, Cocaine of *Erythroxylon coca*, and the Codeine and Morphine of Papaver somniferum, the opium poppy; **Terpenoids** from *Azadirachta indica* (neem tree); **Steroids,** the terpens with a particular ring structure like Saponins; **Glycosides,** the heavily modified sugar molecules like Nojinimycin and Glucosinolates etc.,

Medicinal Plants

Abutilon indicum

A.indicum (Linn) sweet is a hairy herb commonly known as 'Indian Mallow' belonging to family 'Malvaceae' and is called as shikka in Marathi and as Stibala in Sanskrit. *Abutilon indicum* is also known as 'ATIBALA' in Sanskrit. Literally, 'ATI' means 'very' and 'BALA' means 'powerful' referring to the properties of the plant as very powerful. In traditional systems of medicine various plant parts such as leaves, flowers, roots, bark, bark, seeds and stem has been used as anti-oxidant. It is found abundantly in the hotter parts of India but it occurs throughout the tropica, sub-tropica and Ceylon [1,2]. The folk practitioners also use this plant for curing blood dysentery, fever, allergy, aphrodisiac, diuretic problems, toothache, piles, all

kinds of inflammation, bronchitis, gonorrhea, inflammation of bladder and also in leprosy [3].

The plant leaf material have secondary metabolites like mucilage, tannins, organic acids, traces of Asparagine etc., and leaf ash posses magnesium phosphates, calcium carbonate and alkaline sulphates[1]. The leaf also contains sterols, alkaloids, glycosides, titerpenoids, various amino acids as well as essential oils[4]. The root parts have Gallic acid and Asparagin. The presence of terpenoids, sterols, steroids, terpens and flavonoids in roots[5]. Similarly, the incidence of isoalantolatone and alantolatone is also described in the flowers of this plant [6]. Similarly seeds posses the water soluble Galactomannan composed of D-galactose and D-mannose in the ratio of 2:3[7].

Balanites aegyptiaca

Balanites aegyptiaca is a species of tree, classified as a member of the Zygophyllaceae or the Balanitaceae. It is called hinganbet in Marathi and as ingudi or tapsamudrama in Sanskrit. In India it is particular to found in Rajastan, Gujarat, Madhya Pradesh and Deccan. This is one of the most common trees in Senegal. It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay and climate moisture levels [8]. Many parts of the plant are edible like fruit, leaves, seeds and flowers. The fruit can be fermented for the production of alcoholic beverages. Its parts are also used to treat whooping cough and skin diseases, insect bites, cold and itching. Seed oil is applied on brunt area. However, the plant is also having antivenin potential [9]. By the phytochemical screening of methanol and acetone extract of stem, bark, seeds of *B.aegyptiaca*, the presences of alkaloids, flavonoids and glycosides was not detected however Saponins, tannins and volatile oils were detected [10].

Caesalpinia bonduc

This medicinal plant is from the family Caesalpiniaceae and is known as Nata Karanja in Hindi and as fever nut, Bonduc Nut in English, Nata Karanja in Hindi, fever nut, Bonduc Nut in English, Gajga or sagargota in Marathi and as kantki karang or putgi kanrag in Sanskrit.

Diabetes mellitus is a key syndrome typified by derangement in protein, fat and carbohydrate metabolism. Besides hyperglycemia numerous other factors like enhanced oxidative stress and hyperlipidaemia play a crucial role in diabetes. Though, many artificial drugs are existing in the market, diabetes and its allied impediments still stay uncontrolled. The medicinal plants have been used since early days to cure diabetes and its associated problems[11]. Herbal ingredients are prescribed as drugs very extensively due to their relative low cost, effectiveness and lesser side effects though the active biological compounds present in them are unknown. The young leaves and twigs of *Caesalpinia bonduc* are traditionally used for the treatment of Diabetes, tumors, inflammation, and liver disorders. The other medicinal uses of this plant includes treatment of malaria fever, rheumatism, wound healing, tympanitis, stomach pain, dysentery, diarrhea, indigestion, control of intestinal worms and swelling [12].

In addition, various parts of this plant has been reported to possess multiple therapeutic properties like anti-diabetic, antipyretic, antibacterial, antidiuretic, anticonvulsant, anthelmintic, antiviral anti-asthmatic, antiamoebic, and anti-estrogenic activities [13]. All parts of the plant have medicinal properties [3].

MATERIALS AND METHODS

Plant material Survey and Phytochemical Extraction

Ethno medicinal survey was carried out to document the precious indigenous health care practices prevalent among the different ethnic groups i.e., Tirumala, Khygal, Chittor, Puttur, Tirupathi of Chittor district, Andhra Pradesh, India. The tribal's belonging to primitive or aboriginal culture possess a good deal of information about medicinal utility and bio-diversity. Indigenous health care practioners provide low cost alternatives in the situation where the modern health care services are not available or too expensive. The plant materials namely *A.indicum*, *B.aegyptiaca* and *C.bonduc* are collected from the forests of Chittor district of Andhra Pradesh. The same was identified by Dr. K. Madhava Chetty, Department of Botany, S. V. University, Tirupathi.

The fresh, healthy, disease free plant materials of three selected plants were washed under running tap water. The plant materials like leaves, stem, flowers, fruits and seeds were separated and shade dried with occasional shifting. After drying at 37℃ temperature dried plant material were powdered with the help of a grinder machine. Exposure to direct sunlight was avoided to prevent the loss of active compounds; these powdered materials were seived through muslin cloth and stored in air tight bottles for chemical analysis, anatomical analysis of powdered drugs and physico-chemical analysis. Selected powdered plant material was subjected to soxhlet extraction with petroleum ether (60-80℃), methanol (64.5-65.5℃) and water for 3-4 hours in the order is increasing polarity of solvents. The collected solvents were evaporated to make its final volume one fourth of the original volume. The extracts were stored at 4℃ in air tight bottles for further study. Fresh parts were stored in 70% alcohol.

Preliminary Phytochemical screening of selected plants

The preliminary phyto-chemical screening was performed by using plant extract according to Johnsen [14], Gurr [15], Harborne [16], Edeogaet [17] and Krishnaiah [18].

Detection of Inorganic constituents

To freshly prepared ash of plant was added to 50% $^{v}/_{v}$ HCl. It was kept for 1-2 hrs and filtered. The filtrate was used for chemical tests for detection of inorganic constituents like Calcium, Magnesium, Sodium, Potassium and Iron.

Physico-chemical Analysis

Determination of moisture of crude drug

About 1.5g powdered drug was weight in thin porcelain dish (IW). It is dried in oven at 100^{o}c to 105^{o}c, cooled in desiccators and weight and dry weight way taken (DW). The moisture contents were determined by the formula.

Moisture contents = (IW-DW)/IW * 100

Determination of Ash values of crude drug

Ash values are used to determine quality and purity of a drug. Ash contains inorganic radical like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalate, silica, carbonate contents of the crude drug affects total ash value. Total ash, acid-insoluble ash and water soluble values were determined for the drugs.

Determination of extractive values

Extractive values of drug plant materials are useful for the evaluation of a crude drug. It gives idea about the nature of the chemical constituents present in the crude drug which is also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. Water soluble and alcohol soluble extractive values were determined.

Thin layer chromatography

n-butanol, Glacial acetic acid and water is mixed in the ratio of 4:1:5 served as the mobile phase. Standard amino acids were prepared by dissolving the free amino acids tyrosine and phenylalanine in distilled water at a concentration of 1mg/ml 0.05N HCL. Tryptophan is dissolved in dilute (0.05N) NaOH. Unknown sample extraction was done by grinding a known quantity of the sample material in a pestle and mortar with 10 fold volume of 70% ethanol. 100mg Ninhydrin in 100ml acetone or 0.3% Ninhydrin in Butanol containing 3ml of acetic acid served as the Ninhydin reagent.

Antimicrobial assay

About half of the all pharmaceuticals available in the world market are derived from plants [19,20] plants harbor a variety of compounds and most of which are secondary metabolites and aromatic in nature [20]. The compounds are structurally diverse like phenolics, alkaloids, tanins, coumarins and flavanoids which exhibit antimicrobial activity against various microorganisms including fungi, bacteria, viruses and protozoa. The activity of these compounds is different for example leading to the modulation of enzyme activity [19,21]. Quinines are known to interact with amino acids in the microbial proteins like all surface and membrane proteins and modify their functions [19,22]. Flavonoids are the hydroxylated phenolic compounds present in the c6-c3 unit linked to aromatic rings are known to act by complexing with extra cellular as well as soluble protein and may disrupt microbial membrane [23]. Tannins are known to complex with cell surface and soluble proteins and polysaccharides. Alkaloids are exerting toxicity in microorganisms by various mechanisms. For example Barberine intercalates with DNA [24]. *A.indicum, B.aegyptiaca, C.bonduc*, were selected for to test antimicrobial efficacy on the basic of indigenous traditional knowledge.

Authentic pure cultures of human pathogenic bacteria and fungi were obtained from microbial type culture from department of life sciences, S.V. University Tirupathi. *E.coli* SRTCC(3260), *Pseudomanos aeruginosa* SRTCC(708), *Staphylococcus aureus* SRTCC(1073), *Candida albicans* SRTCC(3971) were used as test organisms. Test microorganisms are maintained on nutrient agar slants at 4℃. The slants were incubated at temperature 37℃ for 24 hours and organisms were stored under refrigerator conditions.

Antimicrobial activity of petroleum ether, methanolic and aqueous extract (12.5, 25, 50,100 mg/ml) of the plant sample was determined by using agar well diffusion method. Nutrient agar was used for growth of bacterial strain and potato dextrose agar was used for the growth of fungi.

Plants extracts were dissolved in DMSO at the concentrations of 12.5,25,50,100 mg/ml. The standard anti-microbial solution containing 20µg/ml Floconazole and Ampicillin were prepared. For each bacterial strain control were maintained where pure solvents were used instead of the extract. Each plate was inoculated with 20µg/ml microbial suspension having a concentration of $1*10^8$ cells/ml. test microorganisms were spread on solid plate with the help of sterile swab moistened. A well is made in sterile nutrient agar plate using cork borer. 0.1 ml extract was added to each well the procedure was reported on the test micro-organisms using the standard anti-fungal Fluconazole and antibiotic Ampicillin. The plate containing bacteria were incubated at 37℃ for 24 hours and those containing fungi were incubated at 25℃ for seven days. The positive anti-microbial activity was read based on growth of incubation zone and compared with the standard drug. MIC values were also studied for microorganisms, which were determined as sensitive to the extract in agar well diffusion assay.

MIC was determined by dilution method as described by the National Committees for clinical laboratory standards. The culture was diluted in nutrient agar at 10^6 density adjusted to turbidity of 0.5 Mac Farlans standards. Equal volume of each extract (by serial dilution form there suspension of hot aqueous, methanol and ethanol extract stock solution) and nutrient broth were mixed in test tubes specifically 0.1 ml of standardized inoculums ($5*10^5$CFU/ml) was added to each tube. The tubes were incubated at 37^oc for 24 hours. Two control tubes were maintained for each test batch [25].

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary pytochemical screening of leaf of *Abutilon indicum*, seed of *Balanites aegyptiaca* and *Caesalpinia bonduc* has shown the following results

S.No.	Phytochemicals	*A.indicum*	*B.aegyptiaca*	*C.bonduc*
1.	Alkaloid	-	+	-
2.	Glycoside	-	+	-
3.	Flavonoids	+	-	+
4.	Tannins	+	-	-
5.	Reducing sugars	-	+	+
6.	Phlobatannins	-	-	-
7.	Saponins	+	-	+
8.	Terpenoids	-	+	-
9.	Anthraquinones	-	+	+
10	Cardiac glycosides	-	+	-

Table 1: Preliminary phytochemical analysis of selected plants.

Detection of inorganic constituents

The inorganic composition of leaf samples of *Abutilon indicum*, seed of *Balanites aegyptiaca* and *Caesalpinia bonduc* has shown the following results

S.No.	Inorganic constituents	*A.indicum*	*B.aegyptiaca*	*C.bonduc*
1.	Calcium	+	+	+
2.	Magnesium	+	-	-
3.	Sodium	-	+	+
4.	Potassium	+	+	+
5.	Iron	-	+	+

Table 2: Inorganic composition of leaf samples of selected plants.

Physicochemical analysis

The physico chemical analysis of leaf of *Abutilon indicum*, seed of *Balanites aegyptiaca* and *Caesalpinia bonduc* shows the following results

Parameter	***A.indicum***	***B.aegyptiaca***	***C.bonduc***
Moisture content	11.2%	3.4%	14.5%
Total ash	14.7% of dry wt.	5.2% of dry wt.	11.7% of dry wt.
Acid insoluble ash	5.1% of total ash	2.3% of total ash	3.7% of total ash
Water soluble ash	7.3% of total ash	4.1% of total ash	6.2% of total ash
Water soluble extractives	25.11% of dry wt.	17.5% of dry wt.	29.6% of dry wt.
Alcohol soluble extractive	15.2% of dry wt.	9.2% of dry wt.	14.1% of dry wt.

Table 3: Physicochemical analysis of selected plants.

Thin layer chromatography

The separation of amino acid acids either they occur in the free amino acid pool or as they occur in different protein hydrolysates. The thin layer chromatography is the best known technique of plant biochemistry. The separation and confirmation of unknown amino acid can be confirmed by TLC method. The TLC was sensitive to salt and sugars present in plant extract. Therefore, for separation of amino acids by TLC, the purified plant extract is required.

Thin layer chromatography of amino acid of *Abutilon indicum*

TLC of *A.indicum* is shown in the table. Three spots were observed with different colour and Rf values determined by measuring the distance travelled by solvent and distance by the solute. The spot 1, 2 and 3 with violet colour and the Rf values are 0.31, 0.42 and 0.45 confirms the presence of Valine, Isolucine and Leucine respectively. Thus, three types of amino acids were separated by through TLC.

Spot No.	Colour of spot	Rf value	Standard Rf value	Amino acid	Standard amino acid
1	Violet	0.31	0.32	Valine	Valine
2	Violet	0.42	0.43	Isoleucine	Isoleucine
3	Violet	0.45	0.44	Leucine	Leucine

Table 4: TLC of amino acid of *A.indicum*.

Thin layer chromatography of amino acid of *Balanites aegyptiaca*

TLC of extract of *Balanites aegyptiaca* is shown in the table. Two spots were observed with different colour. The spot 1and 2 are with orange brown and violet colour and the Rf values are 0.34 and 0.43 confirms the presence of Methionine, Isolucine respectively. Thus, two types of amino acids were separated by through TLC.

Spot No.	**Colour of spot**	**Rf value**	**Standard Rf value**	**Amino acid**	**Standard Amino acid**
1	Orange Brown	0.34	0.35	Methionine	Methionine
2	Violet	0.43	0.43	Isoleucine	Isoleucine

Table 5: TLC of amino acid of *Balanites aegyptiaca.*

Thin layer chromatography of amino acid of *Caesalpinia bonduc*

TLC of extract of *Caesalpinia bonduc* is shown in the table. Two spots were observed with different colour. The spot 1and 2 are with brown and violet colour and the Rf values are 0.34 and 0.43 confirms the presence of Methionine, Isolucine respectively. Thus, two types of amino acids were separated by through TLC.

Spot No.	**Colour of spot**	**Rf value**	**Standard Rf value**	**Amino acid**	**Standard Amino acid**
1	Brown	0.34	0.35	Methionine	Methionine
2	Violet	0.45	0.44	Leucine	Leucine

Table 6: TLC of amino acid of *Caesalpinia bonduc.*

Anti-Microbial assay

Antimicrobial activity of various extracts was tested. Majority of the extracts showed inhibitory effect. It was found that all the strains were sensitive to the standard drugs.

Anti-microbial activity of *Abutilon indicum*

The maximum inhibitory activity was shown in 100mg/ml concentration of distilled water against *E.coli* and *C.albicans*, the zone of inhibition is 9.2 mm and 10.2 mm respectively. Whereas, considerable inhibitory activity was observed in 25mg/ml concentration of distilled water against *S.aureus.* The zone of inhibition is 12mm (Table 7). The methanol extract exhibit maximum inhibitory activity in 25mg/ml concentration against *P.aeruginosa*, the zone of inhibition is 10mm. whereas the considerable inhibitory activity was observed at 100mg.ml concentration against *E.coli, S.aureus and C.albicans*. The zones of inhibition are 6.5mm, 9.1mm and 11.2mm respectively (Table 8).

The maximum inhibitory activity was observed in 25mg/ml and 50mg/ml concentration of petroleum ether extract against *C.albicans* and *E.coli*, the zones of inhibition are

9mm and 9.2mm respectively. Whereas, remarkable inhibitory activity was observed at 100mg/ml concentration of petroleum ether extract against *P.aeruginosa* and *S.aureus*, the zones of inhibition are 4.2mm and 5.2mm respectively (Table 9).

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	8.1	9	9.1	**9.2**	7.4	00
P.aeruginosa	7.1	**8**	7.1	7.2	5.5	00
S.aureus	11.2	**12**	10.1	11.2	7.5	00
C.albicans	9.1	9.1	10	**10.2**	8.3	00

Table 7: Anti microbial activity of distilled water extract of *A.indicum*

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	6	6	6.1	**6.5**	4.3	00
P.aeruginosa	9.1	**10**	8.2	9	8.7	00
S.aureus	8	8.1	9	**9.1**	5.4	00
C.albicans	10	10.1	11	**11.2**	6.1	00

Table 8: Anti microbial activity of Methanol extract of *A.indicum*

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	8	9.1	**9.2**	9.1	5.2	00
P.aeruginosa	3	4.1	4	**4.2**	4.1	00
S.aureus	4	4.1	5	**5.2**	4.2	00
C.albicans	8.1	**9**	8.1	8.4	6.2	00

Table 9: Anti microbial activity of petroleum ether extract of *A.indicum*

Anti-microbial activity of *Balanites aegyptiaca*

The distilled water extract shows the maximum inhibitory activity in 100 mg/ml concentration against *E.coli* and *P.aeruginosa* the zones of inhibition are 10.2 mm and 4.3 mm respectively. Whereas, more inhibitory activity was observed at 25 mg/ml concentration of distilled water extract against *S.aureus* and *C.albicans*. The zones of inhibition are 13mm and 16 mm respectively.

The maximum inhibitory activity was observed in 100 mg/ml concentration of Methanol extract against *E.coli*, the zone of inhibition is 4.4mm. Whereas the considerable inhibitory activity was observed in 12.5 mg/ml concentration of methanol extract against *C.albicans*, the zone of inhibition is 12 mm.

The petroleum ether extract does not show any growth of inhibition against selected micro-organisms. However, MIC value is 50 mg/ml for distilled water extract of *B.aegyptiaca* against *P.aeruginosa* while *E.coli*, *S.aureus* and *C.albicans* show 25 mg/ml MIC value. MIC value is 50 mg/ml for Methanol extract against *E.coli* while *C.albicans* show 12.5 mg/ml MIC value.

Microorganisms	Zone of inhibition in					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	9	10	10	**10.2**	8.1	00
P.aeruginosa	4	3	4	**4.3**	2.6	00
S.aureus	12	**13**	12	12.2	9.3	00
C.albicans	15	**16**	15.1	15.3	14.13	00

Table 10: Antimicrobial activity of distilled water extract of *B.aegyptiaca*

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	3	3.1	4	**4.4**	3.5	1.3
P.aeruginosa	0	0	0	0	10.3	00
S.aureus	0	0	0	0	8.1	00
C.albicans	**12**	11	10	10.3	9.9	1.5

Table 11: Antimicrobial activity of methanol extract of *B.aegyptiaca*

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	0	0	0	0	11	00
P.aeruginosa	0	0	0	0	10.3	1.2
S.aureus	0	0	0	0	14.3	00
C.albicans	0	0	0	0	10.1	00

Table 12: Antimicrobial activity of petroleum ether extract of *B.aegyptiaca*

Anti-microbial activity of *Caesalpinia bonduc*

The maximum inhibitory activity observed in 12.5mg/ml concentration of distilled water extract against *C.albicans.* The zone of inhibition is 4mm. whereas considerable inhibitory activity is observed at 100mg/ml concentration of distilled water extract against *E.coli*, *P.aeruginosa* and *S.aureus*. the zones of inhibition are 3.2mm,9.4mm and 5.3mm respectively.

The methanol extract shows the maximum inhibitory activity in 25mg/ml and 50 mg/ml concentration against *S.aureus* and *C.albicans*. The zones of inhibition are 6.1mm and 7mm respectively. Whereas the inhibitory activity was observed at100mg/ml concentration of methanol extract against *E.coli* and *P.aeruginosa*. The zones of inhibition are 3.2mm and 8.1mm respectively.

The maximum inhibitory activity was observed in 100mg/ml concentration of petroleum ether against *C.albicans*. The zone of inhibition is 4.2mm. The petroleum ether extract does not show any growth of inhibition against *E.coli*, *P.aeruginosa* and *S.aureus*.

Microorganisms	Zone of inhibition in					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	2	2.1	3	**3.2**	3.1	00
P.aeruginosa	8	8.1	9.1	**9.4**	9.3	00
S.aureus	4.1	5	5	**5.3**	4.8	00
C.albicans	**4**	3	3	3.3	3.1	00

Table 13: Anti microbial activity of distilled water extract of *Caesalpinia bonduc*

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	2	2.1	3	**3.2**	2.7	1.3
P.aeruginosa	8	7.1	7	**8.1**	5.3	00
S.aureus	5	6	**6.1**	5.9	5.3	00
C.albicans	6	**7.0**	6.1	6.3	3	1.52

Table 14: Anti microbial activity of methanol extract of *Caesalpinia bonduc*

Microorganisms	Zone of inhibition in mm					
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Standard	Control
E.coli	0	0	0	0	6	00
P.aeruginosa	0	0	0	0	11	1.2
S.aureus	0	0	0	0	8.1	00
C.albicans	3	3	4	**4.2**	2.3	00

Table 15: Anti microbial activity of petroleum ether extract of *Caesalpinia bonduc*

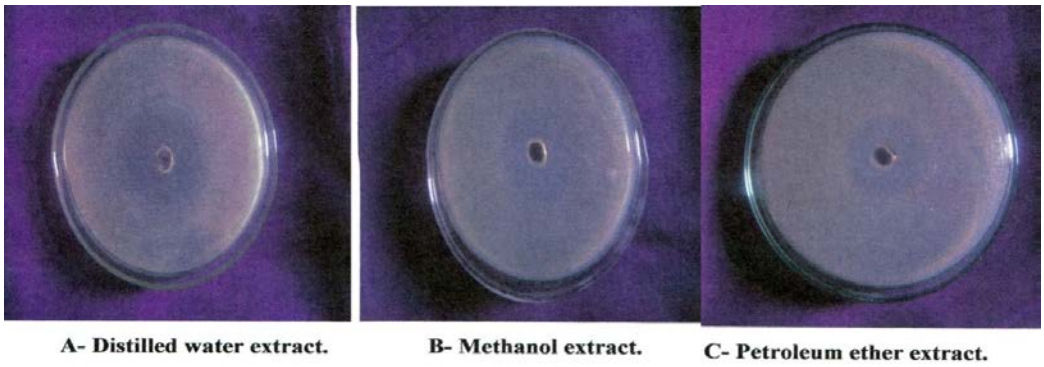

Figure 1: Anti-microbial activity of petroleum ether extract of *A.indicum*

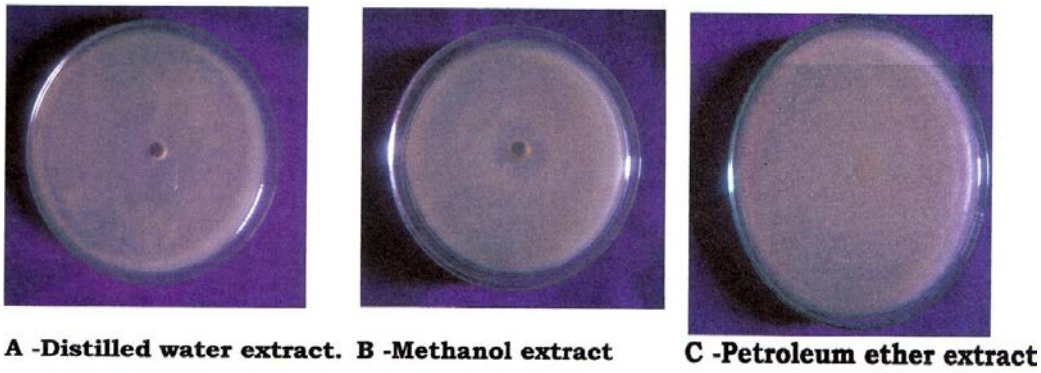

Figure 2: Anti-microbial activity of petroleum ether extract of *B.aegyptiaca*

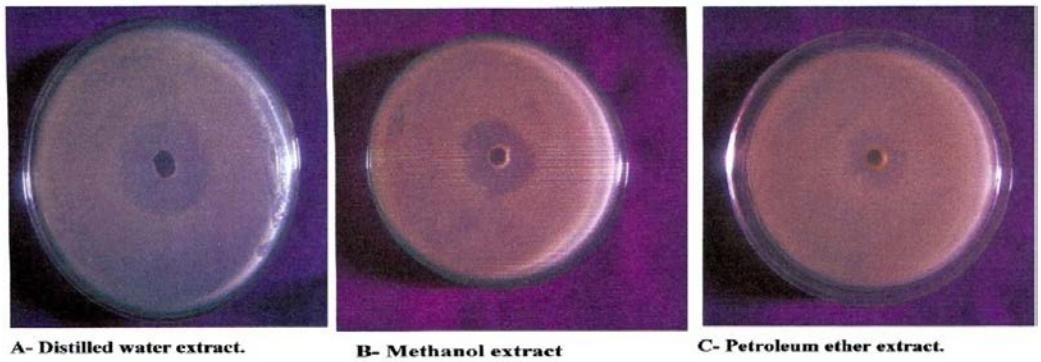

Figure 3: Antimicrobial activity of petroleum ether extract of *C.bonduc*

CONCLUSION

The present study reveals the presence of alkaloids in *B.aegyptiaca*, *A.indicum* and *C.bonduc* which enhance the medicinal potential which reflect in the results of antimicrobial activities against *E.coli*, *P.aeruginosa*, s.aureus and *C.albicans*. Saponins are present in *A.indicum* and *C.bonduc* which exhibit antimicrobial efficacy. Early the efficacy of Saponins of *B.aegyptiaca* was proved against Aedes aegypti larvae [26,27,28]. The study reveals that methanolic extracts of *A.indicum* is more significant against *E.coli*, *P.aeruginosa* and C.albicans as compared to methanolic extracts of *B.aegyptica* and *C.bonduc* with lack tannins. The presence of tannins in *A.indicum* confirms its medicinal potential as anti-diarrheal and potent antibacterial agent. Four types of amino acids are separated by thin layer chromatography technique from plant extracts. Methionine is present in *B.aegyptiaca*, *C.bonduc*. Valine is present only in *A.indicum*. Isolucine is found in *A.indicum*, *B.aegyptiaca*. Leucine is present in *A.indicum* and *C.bonduc*. The present investigation reveals that all plant extracts exhibit activity against the test bacterial strains except the methanolic seed extract of *B.aegyptiaca* and petroleum ether seed extract of *C.bonduc* does not show any anti bacterial activity against test bacteria. The current study noticed that aqueous extracts of *A.indicum*, *B.aegyptiaca* are more potent than the organic solvent extract which support the traditional aqueous decoction methods of tribal's. The results of present study supports the ethno-botanical claims of selected plants and also suggest that these plant extracts possess active compounds having anti-microbial properties that can be used as anti bacterial and anti fungal agent for the evaluation of drugs against disease caused by test microorganisms.

REFERENCES

1. Panda H (1999) Herbs cultivation and medicinal uses. National Institute of Industrial Research New Delhi, India.
2. Chopra RN, Nayar SL (1956) Glossary of Indian medicinal plants. Council of Scientific And Industrial Research; New Delhi.
3. Kirtikar KR (1918) Indian Medicinal Plants Vol-4. Bishen Singh Mahendra Pal Singh; Dehradun.
4. Sharma P, Yelne M, Dennis T, Joshi A (2002) Database on Medicinal Plants Used in Ayurveda & Siddha vol 5. Central Council for Research in Ayurveda & Siddha, Deptt. of ISM & H, Min …
5. Bhattacharjee A, Das A (1969) Phytochemical screening of some Indian plants Quarterly Journal of Crude Drug Research 9:1408-1412.
6. Sharma PV, Ahmad ZA (1989) Two sesquiterpene lactones from Abutilon indicum Phytochemistry 28:3525.
7. Singh V, Mishra UC, Khare GC, Gupta PC (1997) A neutral seed gum from Abutilon indicum Carbohydrate Polymers 33:203-205.
8. Chothani DL, Vaghasiya H (2011) A review on Balanites aegyptiaca Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity Pharmacognosy reviews 5:55.
9. Wufem B, Adamu HM, Cham Y, Kela S (2007) Preliminary studies on the antivenin potential and phytochemical analysis of the crude extracts of Balanites aegyptiaca (Linn.) Delile on albino rats Natural Product Radiance 6:18-21.
10. Sofowora A (1996) Medicinal plants and traditional medicine in Africa. Karthala.
11. Bailey CJ, Day C (1989) Traditional plant medicines as treatments for diabetes Diabetes care 12:553-564.
12. Valiathan M (1998) Healing plants Current science 75:1122-1127.
13. Singh V, Raghav PK (2012) Review on pharmacological properties of Caesalpinia bonduc L Int J Med Arom Plants 2:514-530.
14. Johansen DA (1940) Plant microtechnique Mc Graw-Hill Book Co iInc New York and London 511pp.
15. Gurr E (1965) The rational use of dyes in biology and general staining methods The rational use of dyes in biology and general staining methods.
16. Harborne J (1984) Methods of plant analysis. In: Phytochemical methods. Springer, pp 1-36.
17. Edeoga H, Okwu D, Mbaebie B (2005) Phytochemical constituents of some Nigerian medicinal plants African journal of biotechnology 4:685-688.
18. Krishnaiah D, Devi T, Bono A, Sarbatly R (2009) Studies on phytochemical constituents of six Malaysian medicinal plants Journal of medicinal plants research 3:067-072.
19. Cowan MM (1999) Plant products as antimicrobial agents Clinical microbiology reviews 12:564-582.
20. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL (1993) Unconventional medicine in the United States--prevalence, costs, and patterns of use New England Journal of Medicine 328:246-252.
21. Geissman T (1963) Flavonoid Compounds, Tannins, Lignins and, Related Compounds. In: Comprehensive biochemistry, vol 9. Elsevier, pp 213-250.
22. Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996) Phlorotannin-protein interactions Journal of chemical ecology 22:1877-1899.
23. Tsuchiya H et al. (1996) Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus Journal of ethnopharmacology 50:27-34.
24. Phillipson J, ONeill M (1989) New leads to the treatment of protozoal infections based on natural product molecules Acta Pharmaceutica Nordica 1:131-144.
25. orgensen JH (1993) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard: NCCLS document M7-A3. NCCLS.
26. Chapagain B, Wiesman Z (2005) Larvicidal effects of aqueous extracts of Balanites aegyptiaca (desert date) against the larvae of Culex pipiens mosquitoes African journal of Biotechnology 4:1351-1354.
27. Pelah D, Abramovich Z, Markus A, Wiesman Z (2002) The use of commercial saponin from Quillaja saponaria bark as a natural larvicidal agent against Aedes aegypti and Culex pipiens Journal of Ethnopharmacology 81:407-409.
28. Zarroug IM, Nugud A, Bashir A, Mageed A (1990) Balanites aegyptiaca as a mosquito larvicide International Journal of Crude Drug Research 28:267-271.