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## Preliminary Phytochemical Analysis and Extraction of Crude Drugs from Medicinal Plants

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**ABSTRACT**: Herbal managements are becoming all the time more popular, as the herbal preparations have no or least side effects than man-made drugs. In our present study, the various parts of medicinal plants are used in Ayurvedic and other general 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 have effective for the treatments of alimentary canal, bronchitis, diarrhea, inflammation, cough, skin, insect bite, abdominal pain, gastrointestinal complaints and for proper regulation of menstrual flow.

KEYWORDS: Phytochemical, medicinal plants, Abutilon indicum, Balanites egyptiaca, Caesalpinia bonduc..

#### I. INTRODUCTION

The use of every existing ways of plant protection, about 33 percent of annual harvested food commodities of the globe are 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



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terpens with a particular ring structure like Saponins; Glycosides, the heavily modified sugar molecules like Nojinimycin and Glucosinolates.

#### II. METHODOLOGY

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. The tribe's belonging to primitive or aboriginal culture possesses 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 Shimoga district of Karnataka.

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°C 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°C), methanol (64.5-65.5°C) 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°C 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 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 100oc to 105oc, 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.



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#### III. EXPERIMENTAL RESULTS

#### **Preliminary Phytochemical Screening**

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

Sl.	Phytochemicals	A.indicum	B.aegyptiaca	C.bonduc
No.				
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

S1.	Inorganic	A.indicum	B.aegyptiaca	C.bonduc
No.	constituents			
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 drywt.	5.2% of dry wt.	11.7% of drywt.
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 drywt.
Alcohol soluble extractive	15.2% of drywt.	9.2% of dry wt.	14.1% of drywt.

Table 3: Physicochemical analysis of selected plants.



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#### 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	Colour	Rf	Standard	Amino	Standard
No.	ofspot	value	Rfvalue	acid	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 1 and 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 1 and 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	StandardRf 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*.

#### IV. CONCLUSION

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.



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