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Physico-Chemical Analysis and Phytochemical Screening of So me Indian Medicinal Plants

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Abstract
Inflammation is the body defense reaction to eliminate or limit the spread of an injurious agent as well as to remove the consequently necrosed cells and tissues. Nonsteroidal and steroidal drugs are generally used to treat inflammation. However, these drugs have side-effects like nausea, vomiting, etc. This led us to search for new anti-inflammatory agents from natural sources, which would be effective and safe. Commiphora wightii, *Solanum xanthocarpum and Sarcostemma acidum* W.&A has many therapeutic uses mentioned in Ayurveda and therefore we aimed to study its anti-inflammatory activity both alone and in combination. The extraction of Commiphora wightii, *Solanum xanthocarpum and Sarcostemma acidum* W.&A.was carried out with water, ethanol, chloroform, ethyl acetate and petroleum ether by using soxhlet apparatus. The extracts were screened for the presence of various medicinally active constituents. Physicochemical studies revealed that major active constituent are present in ethanol solvent.
Keyword – Inflammation, side-effects,extraction, ethanol, Commiphora wightii, active constituents.

Introduction –

Natural products from plants, animals and minerals are the basis for treating human diseases. Medicinal plants are presently in demand and their acceptance is increasing progressively. Undoubtedly, plants play an important role by providing essential services in ecosystems. The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, skin, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms.

Inflammation is a complex response to local injury or other trauma; it is characterized by redness, heat, swelling, and pain. Nonsteroidal and steroidal drugs are generally used as a part of drug therapy in inflammation. However, these drugs have severe side-effects like nausea and vomiting. Therefore, there is a need to identify anti-inflammatory compounds that will be effective with a better safety profile. Many plant extracts show a synergistic effect with each other or with modern drugs. The extracts of Commiphora wightii, *Solanum xanthocarpum and Sarcostemma acidum* W.&A have been reported to possess anti-inflammatory activity. We conducted this study to determine whether both these plant extracts showed increased anti-inflammatory activity at low dose when given in combination. [1-6]

Material & Methods

1. Collection of plant material and Preparation of plant powder

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The plant Commiphora wightii, *Solanum xanthocarpum and Sarcostemma acidum* W.&A. were collected from Jabalpur and was authenticated. The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

2. Physico-Chemical Analysis

The powdered plant material of was subjected to standard procedure for the determination of various physicochemical parameters.

Determination of ash values

Ash value is measured to evaluate low-grade material, chemical waste and , exhausted drugs and earthy matter or sandy . Water-soluble ash and acid-insoluble ash can also be used as chemical research.

Total ash value

Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

Acid insoluble ash

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Determination of moisture content (Loss on drying)

About 10 g of drug (without preliminary drying) after accurately weighing was placed in a tared evaporating dish and kept in oven at 105^0 C for 5 hours and weigh. The percentage loss on drying with reference to the air dried drug was calculated.

Determination of foreign organic matter

Accurately weighed 100 g of the drug sample was spread in a thin layer. The foreign matter was detected by inspection with the unaided eye by the use of a lens (6X). The foreign matter was separated and weighed and the percentage present was calculated.

Determination of swelling index

Swelling index is determined for the presence of mucilage. Accurately weighed 1 g of the powdered plant part was placed in 150 ml measuring cylinder. To this 50 ml of distilled water was added and kept aside for 24 hours with occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured. Dwivedi et.al. [7-8]

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3. Preparation of extracts:

The dried powder of plant was extracted with various solvents. Aqueous extract was prepared by cold maceration process. Ethanolic, chloroform, petroleum ether and ethyl acetate extract were obtained using Soxhlet apparatus.

About 250 gm of *S. xanthocarpum* dried fruit, 250 gm of Commiphora wightii and 250 gm of dried powder stem of plant was subjected to soxhlation. It was first defatted with petroleum ether then exhaustively extracted with solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.[7]

4.Phytochemical Screening of S. brevistigma W. & Ar.[8-12]

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extracts.

Tests for carbohydrates and glycosides

Molisch's test

Sample was treated with 2-3 drops of 1% alcoholic - napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Legal's test

To the sample 1 ml of pyridine and few drops of sodium nitroprusside solutions was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

- Dragendroff's reagent - Reddish brown ppt
- Wagner;s reagent - Reddish brown ppt
- Mayer's reagent - Cream color ppt
- Hager's reagent - Yellow color ppt

Test for proteins and free amino acids

Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

- Million's reagent: Appearance of red color shows the presence of protein and free amino acid.
- Ninhydrin reagent: Appearance of purple color shows the presence of Proteins and free amino acids
- Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

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Test for tannins

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Violet color.
- 10% lead acetate solution - White precipitate

Test for flavonoids

Alkaline reagent test

To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test

Small quantities of the sample was dissolved in alcohol, to this piece of magnesium followed by concentrated hydrochloric acid drop wise added and heated. Appearance of magneta color shows the presence of flavonoids**.**

Tests for fixed oils and fats Spot test

- A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.
- Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolpthlein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for steroids and triterpenoids

Libermann-burchard test

Sample was treated with few drops of acetic anhydride, boils and cooled. Then concentrated sulphuric acid was added from the side of test tube, brown ring was formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

Salkowski test

Sample was treated with few drop of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for mucilages and gums

Small quantities of sample was added separately to 25 ml. of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

Test for waxes

To the test solution alcoholic alkali solution was added, the waxes get saponified.

Result And Discussion

1.Physico-Chemical Analysis

The dried parts of *plants* were subjected to standard procedure for the determination of various physicochemical parameters- ash values (total ash, acid insoluble ash and water soluble ash) and Loss on drying were determined.(Table.1)

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Table 1 (a): Physico-chemical analysis of *Sarcostemma brevistigma*

Sr. No.	Parameters	Values (%)
1.	Total ash	7.9
2.	Loss on drying at110°C	1.7
3.	Water soluble ash	1.8
4.	Acid in soluble ash	1.6

Table 1(b): Physicochemical parameters of *Solanum xanthocarpum fruits*

Sr. No.	Parameters	Values (%)
1.	Total ash	8 .1
	Acid insoluble ash	3.9
3.	Water soluble ash	3.5
4.	Loss on drying at110°C	5.3

Table 1(c): Physicochemical parameters of *Commiphora wighii gum resin*

Sr. No.	Parameters	Values (%)
1.	Total ash	12.21
	Acid insoluble ash	7.43
3.	Water soluble ash	5.06
4.	Loss on drying at110°C	7.5

6.2 Extraction

The dried powder of plant was extracted with various solvents i.e., water, ethanolic, chloroform, petroleum ether and ethyl acetate. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract. The percentage yields of various extract was presented in Table 2.

6.2.1 Phytochemical Screening

The various extracts obtained were subjected to preliminary phytochemical screening. The extraction was carried out with water, ethanol, chloroform, ethyl acetate and petroleum ether the extract were screened for the presence of various medicinally active constituents. Aqueous extract shows presence of alkaloids, carbohydrates, glycosides, tannins, protein, amino acids, steroids. Ethanolic extract shows presence of alkaloids, carbohydrates, glycosides, tannins, protein, amino acids, steroids. Chloroform extract shows presence of carbohydrates, tannins while petroleum ether extract shows presence of alkaloids, carbohydrates, glycosides, protein and amino acids, steroids.

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Table 2(a): Extractive value of different extract of *S. brevistigma*

S/No.	Type of Extract	% Yield (w/w)	Color of Extract
1.	Aqueous extract	3.974	Light Green
2.	Ethanolic extract	11.965	Dark Green
3.	Chloroflam extract	09.561	Light Green
4.	Pet. Ether extract	8.943	Brownish Green
5.	Ethyl acetate extract	16.651	Dark Green

Table 2(b): Extractive values of *Solanum xanthocarpum*

S/No.	Type of Extract	% Yield (w/w)	Color of Extract
1.	Aqueous extract	18.01	Light Yellow
2.	Ethanolic extract	15.3	Yellow
3.	Chloroflam extract	6.87	Light Yellow
4.	Pet. Ether extract	2.68	Light Yellow
5.	Ethyl acetate extract	12.45	Yellow

Table 2(c): Extractive values of *Commiphora wighii gum resin*

S/No.	Type of Extract	% Yield (w/w)	Color of Extract
1.	Aqueous extract	59.67	Light brown opaque
2.	Ethanolic extract	55.49	Brown translucent
3.	Chloroflam extract	44.87	Brown translucent
4.	Pet. Ether extract	09.65	Brown translucent
5.	Ethyl acetate extract	17.98	Brown translucent

Since, the major active constituents are present in ethanolic extract therefore; the ethanolic extract will be taken for further investigation. The results are shown in Table 3.

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Table 3(a): Preliminary phytochemical screening of different extract of *Sarcostigmma brevistigma*

S/No.	Constituents	AE	EE	CE	PE	EA
1.	Alkaloids	+	+	-	+	+
2.	Carbohydrates	+	+	+	+	+
3.	Glycosides	+	+	-	+	+
4.	Fixed oil and fats	-	-	-	-	-
5.	Tannins	+	+	+	-	+
6.	Protein and amino acid	+	+	-	+	+
7.	Flavanoids	-	-	-	-	-
8.	Steroids and triterpenoids	-	-	-	-	-
9.	Mucilage and gum	+	+	-	+	+
10.	Wax	-	-	-	-	-

AE: Aqueous Extract; EE: Ethanolic Extract; CE: Chloroform Extract; PE: Petroleum ether Extract, EA: Ethyl acetate Extract (+ Present, - Absent)

Table 3(b): Preliminary phytochemical screening of different extract of *Solanum xanthocarpum*

S/No.	Constituents	AE	EE	CE	PE	EA
1.	Alkaloids	+	+	-	+	+
2.	Carbohydrates	-	+	+	-	-
3.	Glycosides	+	+	+	-	+
4.	Fixed oil and fats	-	-	-	-	-

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5.	Tannins	-	+	+	-	-
6.	Protein and amino acid	+	-	-	-	-
7.	Flavanoids	-	+	+	-	-
8.	Steroids and triterpenoids	-	-	-	-	-
9.	Mucilage and gum	-	-	-	-	-
10.	Wax	-	-	-	-	-

AE: Aqueous Extract; EE: Ethanolic Extract; CE: Chloroform Extract; PE: Petroleum ether Extract, EA: Ethyl acetate Extract (+ Present, - Absent)

Table 3(c): Preliminary phytochemical screening of different extract of *Commiphora wighii gum resin*

S/No.	**Constituents**	**AE**	**EE**	**CE**	**PE**	**EA**
1.	Alkaloids	-	-	-	-	+
2.	Carbohydrates	-	-	-	+	+
3.	Glycosides	+	+	+	+	+
4.	Fixed oil and fats	-	+	-	+	-
5.	Tannins	-	-	-	-	-
6.	Protein and amino acid	-	+	-	-	-
7.	Flavanoids	-	+	-	-	-
8.	Steroids and triterpenoids	-	-	-	-	-
9.	Mucilage and gum	+	+	+	+	+
10.	Resins	+	+	+	+	+

AE: Aqueous Extract; EE: Ethanolic Extract; CE: Chloroform Extract; PE: Petroleum ether Extract, EA: Ethyl acetate Extract (+ Present, - Absent)

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