Phytochemical Testing To Discover Therapeutic Value of Plants

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Abstract: - Since the beginning of human civilization, medicinal plants has been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder form after removing the solvent, and are intended for oral or external use. In this study Phytochemical testing of the medicinal plants have been done. The objective here is to do a qualitative and quantitative analysis of plant extracts and to determine the antibacterial properties of the same. Plants used are, Arjuna (*Terminalia arjuna*), Ajwain (*Trachyspermum ammi*), Gokhru (*Tribuls terristris*).

Key Words: — ***Human civilization, medicinal plants, modern drugs, selective solvents, Phytochemical testing***.

I. INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents [2]. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The purpose of standardized extraction procedures for crude drugs is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstruum [3]. The basic parameters determining the quality of an extract are [4]:

- Plant part used as starting material
- Solvent used for extraction
- Extraction procedure

Effect of extracted plant phytochemicals depends on [5]:

- The nature of the plant material
- Plant material origin
- Degree of processing
- Moisture content
- Particle size

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. i.e. any part of the plant may contain active components[6].

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II. MATERIALS AND METHODS

A. Plant Collection

Botanical name	Common name	Solvent used	Plant part used	Obtained from
Terminalia arjuna	Arjun	Ethanol	Bark	College campus
Trachyspermum ammi	Ajwain	Ethanol	Leaves	Nursery
Tribuls terristris	Gokhru	Ethanol	Leaves	Nursery

B. Extract Preparation

The shade dried and coarsely powdered (10 g) Arjun was subjected to Steam distillation, using methanol. The extracts were then concentrated to dryness under reduced pressure and controlled temperature.

The shade dried and coarsely powdered (10 g) Ajwain was subjected to Steam distillation, using methanol. The extracts were then concentrated to dryness under reduced pressure and controlled temperature.

The shade dried and coarsely powdered (10 g) Gokhru was subjected to Steam distillation, using methanol. The extracts were then concentrated to dryness under reduced pressure and controlled temperature.

C. Qualitative Estimation

Qualitative Screening for phytochemical testing was carried out for all the extracts as per the standard methods.

- Terpenoids: 2 ml Chloroform was added to 5 ml aqueous sample along with 3 ml conc. H2SO4. Greyish colour development indicates the presence of terpenoids.
- Saponins: 1ml sample was taken and added to 4 ml distilled water. The solution was vigorously shaken for a minute and then the system was left to rest for 15 minutes. Persistence of froth indicates the saponins presence.
- Philobatannins: 2 ml aqueous sample was taken and 1% HCL was added to it followed by the boiling of the resultant solution. A red precipitate marks the presence of philobatannins.
- Carbohydrates: 1 ml aqueous sample was taken and a few drops of Fehling's solution were added to it. The solution was then incubated at 4°C for 5 minutes. Appearance of a red colour indicates carbohydrates.
- Tannins: 0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride (FeCl3) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test sample.
- Phenols: In 1 ml of various solvent extracts of sample, 2 ml of distilled water was added followed by a few drops of 10% aqueous ferric chloride solution. Formation of blue or green colour indicated the presence of phenols.
- Alkaloids (Wagner's test): Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.
- Proteins and Amino acids (Ninhydrin test): 0.25% w/v ninhydrin reagent was added in the extract and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.
- Flavonoids (Alkaline reagent test): Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of Flavonoids.

D. Quantitative Estimation

Total Phenol Content: Phenolic compounds are a class of antioxidant compounds which act as free radical terminators. The ethanolic extract of three plants was studied for their content of total phenols. The total phenolic content of extract is measured by folin-ciocalteau reagent in terms of gallic acid equivalent (GAE).

Determination of Antibacterial Properties: Antibacterial activities of plant extract were investigated by the disc diffusion method. The sensitivity of the microbes species to the plant extract was determined by measuring the size of zone of inhibition (including diameter of disc), on the agar surface around the disc.

III. Results And Discussion

A. Percentage Yield

The bark of Arjun, leaves of Ajwain and Gokhru were extracted with methanol.

Table.1. Percent yield of plant extracts

S.No	Name of the extract	Yield (%)
1	Arjun	57.76%
2	Ajwain	30.25%
3	Gokhru	62.59%

B. Qualitative Analysis

Table.2. Phytochemical Testing of Plant Extracts

Phytochemical	Arjun	Ajwain	Gokhru
Terpenoids	X	X	X
SaponIns	++	++	++
Philobatannins	+	+	X
Carbohydrates	+	+	+
Tannins	++	++	+++
Alkaloids	+++	++	++
Phenols	+++	+++	++
Proteins and Amino acids	+	++	X
Flavanoids	++	++	++

[+]: Compounds present

[X]: Compound absent

[+++]: Present in high amount

[++]: Present in medium amount

[+]: Present in low amount

C. Quantitative Analysis

Total phenol content:

The methanolic extract of three plants was studied for their total phenol content. Values of phenol content were calculated as Gallic Acid Equivalent (GAE) from standard curve of gallic acid.

Table.3. Standard curve of gallic acid

Gallic acid (mg/ml)	Absorbance (760nm)
0.1	0.11
0.2	0.29
0.3	0.32
0.4	0.55
0.5	0.72
0.6	0.85

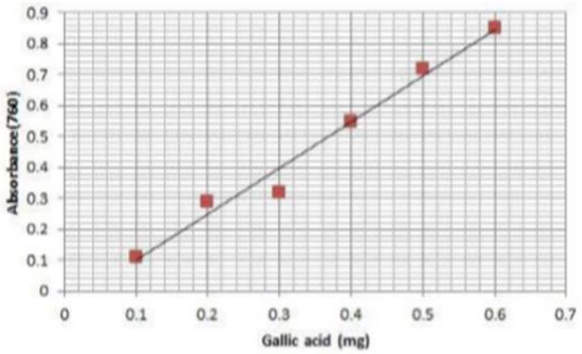

Fig.1. Standard curve of Gallic acid

Table.4. Total phenol content of plant extract

Plant extract	Total phenol (µg GAE/gm extract)
Arjuna	341
Ajwain	320
Gokhru	172

Determination of the Antibacterial properties:

Antibacterial activities of plant extract were investigated by the disc diffusion method. The sensitivity of the microbes species to the plant extract was determined by measuring the size of zone of inhibition (including diameter of disc) on the agar surface around the disc which was found to be maximum for Ajwain in both *E.coli* and *B. Subtilis.*

Table.5. Zone of inhibition of the plant extracts

Plant extract	Zone of inhibition in *Bacillus subtilis* (cm)	Zone of inhibition in *E. Coli* (cm)
ARJUNA	1	0.65
AJWAIN	1.2	0.7
GOKHRU	0.75	0.55

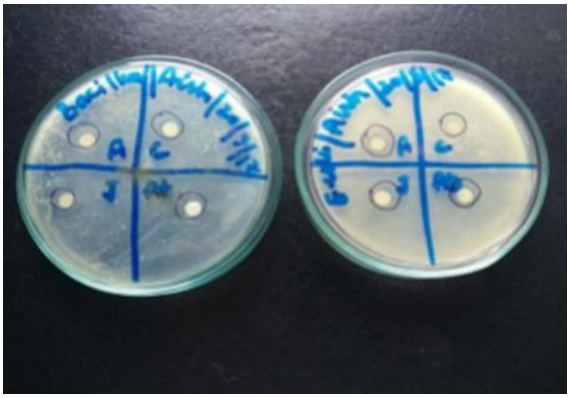

Fig.2. Antimicrobial properties of plant extract by disc diffusion assay

IV. Conclusion

The selected medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. Medicinal plants play a vital role in preventing various diseases. The anti-diuretic, anti-inflammatory, anti-analgesic, anti-cancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. The phytochemical analysis of the medicinal plants is also important and has commercial interest in both research institutes and pharmaceutical companies for the manufacturing of the new drugs for treatment of various diseases.

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