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Comparative analysis of the Phytochemicals present in different extracts of *Operculina turpethum*

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Abstract

Plant kingdom harbours an inexhaustible source of active ingredients valuable in the management of many intractable diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. The plant material was dried in shade, crushed and subjected to prepare different sequential extracts using soxhlet apparatus. Phytochemical screening of various extracts of *Operculina turpethum* was carried out by employing standard methods for conducting Qualitative phytochemical analysis for studying the presence of active compounds like Alkaloids, Tannins, Saponins, Glycosides, Phenols, Flavonoids, sugars, Terpenoids and Steroids. Our findings of crude extracts of the plant revealed the presence of saponins, steroid, alkaloids, terpenoid, flavonoids, cardic glycosides. It equally ascertains the bioactive components in the plant, thus agreeing with the potential therapeutic significance of the plant as a natural source of drug development.

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INTRODUCTION

Recent research on phytochemicals is fervently focusing on health promotion, disease prevention, and the development of therapeutic interventions. The introduction of terms such as "functional food" and "nutraceutical" illustrates the high expectations associated with current phytochemical research [1]. Phytochemistry is a branch of science that deals with

the chemicals obtained from plants with desirable biological activities. The medicinal flora in India has a pre-ponderance of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. The success story of phytotherapy lies in the continuous search for new drugs to counter various health challenges [2]. There is now growing evidence that indicates a strong relationship between ethnic knowledge and sustainable use of biodiversity [3]. The time- tested ethnic knowledge when supplemented with the latest scientific insights can offer new models of economic development, that are both eco-friendly and socially acceptable [4].

The uses of plant extracts, as well as other alternative forms of medical treatments, have enjoyed great popularity in the late 1990s. It is estimated that today, plant materials are present in, or have provided the models for 50% of Western drugs. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments [5]. Hence there are considerable benefits in the development of indigenous medicines and in the use of medicinal plants for treatment of various diseases [6]. Traditional knowledge of medicinal plants and their uses by indigenous cultures are not only useful for conservation of cultural traditions, but also for community healthcare and drug development in the present and future [7].

Antioxidants help to prevent the free radical damage that is associated with various diseases. The potentially reactive derivatives of oxygen are known as reactive oxygen species (ROS) e.g. superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals, and play an important role in oxidative damage to various biomolecules including proteins, lipids and DNA, related to the pathogenesis of various important diseases. Secondary metabolites which are one of the bioactive constituents present in plants, have been reported to have strong antioxidant activity. These naturally occurring antioxidants have also been reported to show antimicrobial, anticancer, anti-inflammatory activity [8].

Operculina turpethum or Indian Jalap is widely grown throughout India and it is occasionally cultivated in gardens as an ornament. The root bark of Trivrit is rich in turpethin resin consisting of 10% 'turpethin' which is a glycoside analogue of Jalapine and Convolvulin and also contains Turpethinic acids-A, B, C, D, & E, , volatile oil, albumin, starch, lignin salts, ferric oxide, Scopoleptin, Betulin, lupiol & beta-sitosterol [9]. The roots are bitter, acrid, sweet, thermogenic, analgesic, purgative, carminative, antihelmintic, expectorant, antipyretic, hepatic, stimulant and hydragogue.

Although antioxidant properties have been suggested as the basis of health benefits of phytochemicals, emerging findings suggest a quite different mechanism of action. Many phytochemicals function as toxins that protect the plants against insects and other damaging organisms. However, at the relatively low doses consumed by humans and other mammals these same "toxic" phytochemicals activate adaptive cellular stress response pathways that can protect the cells against a variety of adverse conditions. Recent findings have elucidated the mechanisms of action of phytochemicals using cell culture and animal models of neurological disorders. Examples of these pathways activated by phytochemicals include the transcription factor Nrf-2 which activates genes controlled by the antioxidant response element, and histone deacetylases of the sirtuin family and FOXO transcription factors [10]. Such pathways stimulate the production of antioxidant enzymes, protein chaperones and neurotrophic factors. Hence, the qualitative screening of the plant for the presence of various phytochemicals finds its relevance to provide the source of potent natural remedy.

MATERIALS AND METHODS

Plant Material

Operculina turpethum was collected from Pharmacological garden of CCSHAU Hisar , Haryana, India in the month of august 2012. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra Rohtak, Haryana, India.

Preparation of extracts

Dried powdered materials were placed in the soxhlet thimble to obtain sequential extracts of different solvents ranging from non-polar to polar - petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water by placing them in 250 ml round bottom flask. The materials were refluxed with each solvent for 12- 14 hours at 40-70° C. Extracts were collected and cooled at room temperature and poured in glass Petri dishes & then evaporated at 40°C using hot air oven. Dried extracts were kept in desiccators for two days and stored at 5°C in air tight containers [11].

Yield of extraction

The calculation of the extraction yield was the weight percentage of the crude extract to the raw material (50g). The percent extraction yield was calculated as follows [12]:

% Extraction yield = Weight of the plant extract/ Weight of the initial sample x 100%

Chemicals and Reagents

Ferric chloride, folin-ciocalteu's reagent, gelatin, HCl, dragendorff's reagent, methanol, gallic acid, H_2SO_4, Na_2CO_3, vanillin, tannic acid, acetic anhydride, fehling solutions were all purchased from Merck, USA. All other unlabelled chemicals and reagents were of analytical grade and of high purity.

Qualitative phytochemical screening

The Phytochemical screening of all six extracts was performed by the standard methods [13,14].

Test for alkaloids

a) Mayer's Test- Test solution (1 ml) was taken in test tube and few drops of Mayer's reagent (Potassium mercuric iodide solution) were added into it and cream color precipitate was observed.

b) Dragendroff's Test- Test solution (1 ml) was taken in test tube and few drops of Dragendroff's reagent (Potassium bismuth iodide solution) were added into it and observed for reddish brown precipitate.

c) Tannic acid Test- Test solution (1 ml) was taken in test tube and few drops of 10 % tannic acid solution was added to it and observed for buff coloration.

Test for tannins

a) $FeCl_3$ Test- About 0.5 mg of dried powdered samples were boiled in 20 ml water in test tubes and filtered. A few drops of 0.1 % ferric chloride solution was added and observed for brownish green or blue-black coloration.

b) Gelatin Test- About 1ml test solution was taken in a clean dried test tube and 1 % gelatin solution was added followed by 10 % sodium chloride solution and observed for white precipitate to form.

c) Vanillin hydrochloride Test- Test solution was treated with few drops of vanillin hydrochloride reagent and observed for purplish- red color.

Test for cardiac glycosides

a) Keller killiani Test-Test solution (1 ml) was taken in a test tube and 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added to it. Carefully added 0.5 ml of concentrated sulphuric acid by the side of the test tube and observed for blue color to appear in the acetic acid layer.

b) Salkowski Test- Test solution (1 ml) was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

Test for Steroids

a) Liebermann Buchard test- Test solution of 1 ml was treated with few drops of acetic anhydride, boiled and cooled, concentrated sulphuric acid was added from the sides of the test tube and observed for a brown ring at the junction of the two layers and green layer in upper layer.

Test for Flavonoids

a) Alkaline reagent test- About 1 ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow coloration which disappears on the addition of dilute HCl.

b) Lead acetate Test- Test solution (1 ml) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow colored precipitate.

Test for Terpenoids

a) Salkowski Test- Test solution (1 ml) was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

Test for Proteins

a) Ninhydrin Test- Test solutions were boiled with 0.2 % solution of ninhydrin and observed for violet color to appear.

Test for Reducing sugars

a) Fehling Test- Test sample of 1 ml was taken into a clean and dried test tube and 0.5 ml of Fehling A and Fehling B solutions were added to it, boiled and observed for brick red coloration.

Test for Saponins

a) Froth test- Test solution (1 ml) was placed in a test tube containing water and shaken well and noted for a stable froth that persists for at least 2 min.

RESULTS

Yield of different extracts of *Operculina turpethum* roots.

The yield of plant is mainly dependent on the type of solvent used in the extraction procedure. Table-1 represents the % yield of dried roots in different solvents (non-polar to polar sequentially). The values were high in aqueous extract of roots (30.6%). Among other solvents used for study, petroleum ether has shown minimum extraction yield of 2.4%. After the petroleum ether extract, chloroform extract has shown a little higher value yield of 2.8%. Whereas the benzene, ethyl acetate and ethanol showed the moderate extractive yields as (4.8%, 8.6%, 9%) respectively. Table 1

Table 1: Extraction yield of different extracts of *Operculina turpethum* roots.

Solvents	Yield of extract/50 g roots (%)	
Pet Ether		2.4
Benzene		4.8
Chloroform		2.8
Ethyl Acetate		8.6
Ethanol		9
Aqueous		30.6

SCREENING OF *OPERCULINA TURPETHUM* ROOTS

The present study carried out on the plant samples revealed the presence of medicinally active constituents. Table 2

Table 2: Qualitative phytochemical screening of various extracts of *Operculina turpethum* roots.

Phytochemicals		PEOT	BEOT	CEOT	EAEOT	EEOT	AEOT
Alkaloids	Dragendroff's test	+	-	+	++	++	+++
	Tannic acid t-est	-	+	+	+	-	++
Tannins	Ferric chloride test	+	+	++	+	+	++
	Gelatin test	-	-	-	-	+	++
	Vanillin-HCl test	+	+	++	+	-	+
Cardiac glycosides	Keller-Killiani test	++	+	+++	++	+++	+
	Salkowski test	+	+	+	-	++	+
Steroids	Liebermann-Buchard test	+	-	++	+	++	-
Flavonoids	Alkaline Reagent test	+	+	-	-	+	++
	Lead Acetate test	-	-	+	-	+	+
Terpenoids	Salkowski test	+	++	+	+	++	+
Proteins	Ninhydrin test	-	-	-	-	-	-
Reducing sugars	Fehling's test	++	++	+	++	++	+
Saponins	Froth test	+	+	+	+	+++	+

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. PEOT- Petroleum ether extract of *Operculina turpthum*; BEOT- Benzene extract of*; Operculina turpthum*; CEOT- Chloroform extract of *Operculina turpthum*; EAEOT- Ethyl acetate extract of *Operculina turpthum*; EEOT- Ethanolic extract of *Operculina turpthum*; AEOT- Aqueous extract of *Operculina turpthum.*

DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents is generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for all six extracts showed significant indication about the presence of metabolites. These findings of phytochemicals is good enough to reflect their importance.

As already mentioned, plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. As a matter of fact, many have been shown to present exciting biological and pharmacological activities and may have potential to be used as chemotherapeutic agents or serve as the starting point in the development of new medicines [15]. Plant phenolic compounds especially flavonoids are currently of growing interest owning to their supposed properties in promoting health [16]. In the present investigation, aqueous extract showed the presence of flavanoids which may be accounting for the anti-inflammatory and analgesic activites. The presence of alkaloid in the plant indicates that the plant extracts could be used for the antifungal activity [17]. Saponins, a special class of glycosides, have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature and are reported to have anti-diabetic and anti-fungal properties [18,19].

It has been reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens [20]. Steroids increase nitrogen level in the body, thereby producing proteins that help in the production of muscles. Steroids could enhance metabolism and thus inhibit the accumulation of fat, correct disorders like anemia to increase the production of red blood cells in the body and contribute to the treatment of arthritis,

asthma, brain injury and some types of cancer [21]. The presence of carbohydrates and reducing sugars in the plant seems to indicate the high energy content that could be exploited as a source of raw materials for pharmaceutical industries.

CONCLUSION

The preliminary phytochemical analysis revealed the presence of steroids, reducing sugars, terpenoids, alkaloids, flavonoids, cardiac glycosides, saponins and tannins in different extracts of *Operculina turpethum*. The study apparently highlighted the scientific basis for the possible use of *Operculina turpethum* roots in ethno-medication.

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