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RESEARCH **P**APER

Phytochemical screening of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla)

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The use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. Methanolic extracts of dried leaves of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus emblica* were used for the comparative study of phytochemical constituents. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, saponins, steroids, flavonoids, tannins and reducing sugar. The highest yield of methanolic extract was found in *Azadirachta indica* (29.08%). *Ocimum sanctum* contained all the chemicals except flavonoids and reducing sugar, however, the *Colquhounia coccinea* lacked alkaloids and reducing sugar.

Key words : Phytochemical screening, Alkaloids, Glycosides, Steroids, Flavonoids, Tannins, Reducing sugar

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INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes (Edeoga *et al*., 2005).

Phytochemicals like flavonoids, tannins, terpenoid, saponins are present in the leaves and stem of most of the wild plants (Kayani *et al*., 2007 and Achakzai *et al*., 2009). Similarly the same are also present in *Ocimum sanctum*, while alkaloids are absent. Neem has a multitude of pesticidal active ingradients which are together called "triterpeni" more specifically "limnoids". The four best limnoid compounds are azadirachtin, salannin, meliantriol and nimbin (Mondal and Mondal, 2012). The seeds of *P. emblica* contain fixed oil, phosphatides and small quantity of essential oil. In addition, the leaves contain gallic acid, ellagic acid, chebulagic acid and chebulinic acid. Phyllaemblic acid, a novel highly oxygenated norbisabolane were isolated from the roots of *P.emblica* and its structure was fully characterized by spectroscopic and chemical means (Zhang *et al*., 2003). Ellagic acid and lupeol are present in roots of *P. emblica* (Kapoor, 1990; Rastogi and Mehrotra, 1993). Keeping in view the above importance of medicinal plants, the present study was designed to evaluate the the phytochemical constituent of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem)and *Phyllanthus emblica* (Amla).

RESEARCH METHODOLOGY

Collection of samples :

Ocimum sanctum greenish stem garden of PCSIR

Labs complex Peshawar, brought to the laboratory, washed thoroughly with tap water and shade was taken in volumetric flask, dried at room temperature.

Phytochemical analysis :

Preliminary phytochemical analyses were carried out by the reported procedure (Balasundrum *et al.*, 2005) in which powder sample or extracts of samples (freshly prepared) was treated with different reagents and the result showed the presence of targeted compound.

Determination of alkaloids:

The determination of alkaloids in each sample was carried out by the described method of Harborne (1973). A 50g of sample was well mixed with 10 per cent acetic acid solution in pure ethanol and left for 4 hours at room temperature, after that the mixture was filtered and concentrated to one fourth of its original volume by rotary evaporator. Concentrated NH OH was added drop wise in concentration till the alkaloid was precipitated. The precipitate was collected on weighted filter paper, washed with 1 per cent ammonia, dried in oven at 80°C.

Test for glycosides (Keller Killiani's test) :

When a pinch the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. sulphuric acid, formation of ring at the junction of two liquids indicated the presence of glycosides.

Test for steroids (Salkowski's test) :

About 100 mg extract was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface is indicative of the presence of steroidal ring (Sofowora, 1993).

Determination of flavonoids :

The total flavonoids were also determined by a previous method (Harborne, 1973), accordingly 10 of each sample boiled in 50 ml HCl (2M solution) by reflux condensation for 30 minuets, cooled and filtered. The filtrate was then mixed with equal volume of ethyl acetate. The flavonoids were recovered from the filtrate and the calculated results expressed in mg/g.

Determination of tannins :

Tannins in leaves and stem of *Ocimum sanctum* were determined using 2g of each grinded sample was mixed with 20 ml of 50 per cent methanol, covered with paraffin and placed on water bath at 80°C. After one hour the extract was filtered. 1 ml from extract was taken in volumetric flask, added 20ml distilled water, 10 ml Na CO (17%) and 2.5ml Folin denis reagent and made the volume 50ml with distilled water. A bluish green colour developed after 20 minuets. The absorption was read at 760nm by spectrophotometer with the different concentration 0-10 ppm of tannic acid standard treated as a sample and tannin concentration was calculated in mg/g (Swain *et al.*, 1979).

Test for carbohydrates and reducing sugar :

The small quantities of the filtrate was dissolved in 4ml of distilled water and filtered. The filtrate was subjected to :

Fehling's test :

The extract was treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

Benedict's test :

The extract was treated with Benedict's reagent; appearance of reddish orange colour precipitate indicates the presence of reducing sugar.

Determination of saponins :

Saponins were determined using 1 g of grinded sample taken in 250 ml beaker, added isobutyl alcohol (100ml) and shacked for 5 hours on orbital shaker. After that the mixture was filtered through Whatman No.1 filter paper into beaker, added 20 ml MgCO saturated solution and again filtered to obtain colourless solution. Then took 1 ml of solution in 50ml flask, mixed with 2ml FeCl (5% solution) and made the volume with distilled water. It was allowed to stand for 30 minuets to develop red colour. The absorbance was recorded at 380nm with the different concentration 0-10 ppm standard saponins. The standard solutions were also treated as a sample and the concentration of saponins was also calculated in mg/g (Brunner, 1984).

RESEARCH FINDINGS AND ANALYSIS

Phytochemical analysis of methanol and aqueous extracts of *O. sanctum, A. indica* and *P. emblica*

demonstrated the presence of phytoconstituents like tannin, saponins, flavonoids, glycosides, reducing sugar, steroids and alkaloids. The medicinal value of the plant lies in bioactive phytochemical action on the human body. Some of the most important bioactive phytochemical constituents were alkaloids, flavonoids, tannins, saponins etc compounds reported earlier. Antibacterial properties of several plant extracts have been attributed to some of these secondary metabolites. The results of the phytochemical screening of methanolic and aqueous leaf extract of *O. sanctum,* revealed the presence and absense of alkaloids, steroids, saponins,and tannins compounds (Table 1). The results of qualitative screening of phytochemical components in leaves of *P. emblica* revealed the presence and absense of alkaloids, tannins, saponins and flavonoids (Table 2).

The result showed that the methanolic and aqueous leaf extract of *Azadirachta indica* contains saponins, tannins, glycosides, alkaloids, flavonoids and reducing sugars which is presented in Table 3. The presence of these phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract. Flavonoid has also been reported to have greater potential benefit to human Health.

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh *et al.,* 2005). It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of

Table 1: Qualitative determination of phytochemical groups of methanolic and aqueous leaf extract of *O. sanctum*

Active chemical constituents in plants	Presence or absence in methanolic leaf extract	Presence or absence in aqueous leaf extract
Alkaloids	+	+
Glycosides	–	–
Steroids	+	+
Flavonoids	–	–
Tannins	+	–
Reducing sugar	–	–
Saponins	–	–

Indication: + = presence – = absent

Table 2: Qualitative determination of phytochemical groups of methanolic and aqueous leaf extract of *P. emblica*

Active chemical constituents in plants	Presence or absence in methanolic leaf extract	Presence or absence in aqueous leaf extract
Alkaloids	+	+
Glycosides	–	–
Steroids	–	–
Flavonoids	+	+
Tannins	+	–
Reducing sugar	–	–
Saponins	+	–

Indication: + = presence – = absent

Table 3: Qualitative determination of Phytochemical groups of methanolic and aqueous leaf extract of *A. indica*

Active chemical constituents in plants	Presence or absence in methanolic leaf extract	Presence or absence in aqueous leaf extract
Alkaloids	–	–
Glycosides	+	+
Steroids	–	–
Flavonoids	+	–
Tannins	+	+
Reducing sugar	+	+
Saponins	+	+

Indication: + = presence – = absent

healthcare. The phytochemical analysis of *A. indica* extract had earlier been reported (Kraus *et al.*, 1981).

Phytochemical screening of the laef extract of *A. indica* in the present study also revealed presence of glycosides. However, a glycoside appeared to be the major bioactive component that offers anti-secretory and antiulcer effects (Bandyopadhyay *et al.*,1998 and 2002). Plant glycosides, which are not normally toxic when ingested orally, are known to inhibit chloride transport in the stomach (Machen and Forte, 1979). Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings.

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