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PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATIONS OF SOME MEDICINAL PLANTS OF NEPAL

Himal Paudel Chhetri*, Nisha Shrestha Yogol, Jyoti Sherchan, Anupa K.C., S. Mansoor, Panna Thapa
Department of Pharmacy
Kathmandu University, P.O. Box: 6250, Kathmandu, Nepal

*Corresponding author: himalchhetri@ku.edu.np

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ABSTRACT
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. The aim of the study was to find out the bioactive chemical constituents and to evaluate the antimicrobial activity of the ethanolic extract of traditionally used eight medicinal plants of Nepal. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar. The highest yield of ethanolic extract was found in *Azadiracta indica* (29.08%). *Ocimum santrum* contained all the chemicals except flavonoids and reducing sugar however the *Colquhounia coccinea* lacked alkaloids and reducing sugar. The antimicrobial activities of these plants extract were also observed. The extract of *Rhododendron setosum* and the essential oil of *Eucalyptus globulus* were most effective against *Escherichia coli* and *Staphylococcus aureus* respectively. But the extracts of *Azadiracta indica* and *Elsholtzia fructicosa* were found to be most effective against *Klebsiella* species.

Key words: Phytochemical screening, plant extracts, zone of inhibition

INTRODUCTION
Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C give many references of medicinal plants. One of the remotest works in traditional herbal medicine is "*Virikshayurveda*", compiled even before the beginning of Christian era. "*Rig Veda*", one of the oldest available literatures written around 2000 B.C. mentions the use of Cinnamon (*Cinnamomum verum*), Ginger (*Zingiber officinale*), Sandalwood (*Santalum album*) etc. not only in religious ceremonies but also in medical preparation (Bentley and Trimen, 1980). Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments (Abraham, 1981). The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant (Holiman, 1989).

According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan, Ayyanar and Ignacimuthu, 2006).

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab, Kamalinejad, Ghaderi and Vahidipour, 2003). Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

Nepal is rich in varieties of medicinal plants. Among the 7000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal (Manandhar, 2000). Unfortunately, only few of them are used for their medicinal value. Our approach involved the collection, identification, extraction, phytochemical and antimicrobial evaluation of the eight medicinal plants found in Nepal.

OBJECTIVE

The objective of the study was to find out the phytochemical active constituents and antimicrobial activities of the following plants found in Nepal.

Table 1. Studied Plants

S.No.	Scientific name	Vernacular name	Plants part used	Family
1	*Azadiracta indica*	Neem	Leaves	Meliacae
2	*Colquhounia coccinea*	Sano Tusare	Whole plant	Labiatae
3	*Curcuma longa*	Haledo	Rhizome	Zingiberaceae
4	*Elsholtzia fructicosa*	Chhiuki	Whole plant	Labiatae
5	*Eucalyptus globulus*	Masala	Leaves	Myrtaceae
6	*Ocimum santrum*	Tulsi	Leaves	Labitae
7	*Rhodendron setosum*	Sunpati	Leaves	Ericaceae
8	*Zanthoxylum aromatum*	Timur	Bark	Rutaceae

MATERIALS AND METHODS

Collection and processing of plants

On the basis of available literature, afore mentioned eight plants were collected from different localities of Nepal during the flowering period, dried in shadow, and then powdered. The powdered crude drug was macerated with 80% ethanol. However, hexane was also used as solvent for the extraction of *Azadiracta indica*. The solvent was then evaporated at a constant temperature of 72^0C until a very concentrated extract was obtained. Identification tests for the various chemicals were carried out to test the presence of various chemical constituents.

Identification Tests

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure:

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer’s reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer’s reagent (Evans, 2002).The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer (Siddiqui and Ali, 1997).

Terpenoid and steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids (Siddiqui and Ali, 1997).

Flavonoid

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones (Siddiqui and Ali, 1997).

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling’s solution was added at hot and observed for brick red precipitate.

All the plant extracts were subjected to individual microbiological tests to ascertain their antimicrobial activity against three species of microorganism: *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella species*. The antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition (ZI) exhibited by the extracts.

The tests were carried out by cup plate method and the diameter of the borer used was 6 millimeter (mm). The microorganisms used were the isolates of a local hospital.

RESULTS AND DISCUSSION

The percentage yields of extracts and the phytochemical constituents of the plants are shown in Table 2 and 3 respectively. The highest yield of ethanolic extract was found in *Azadiracta indica* (29.08%) and the lowest in *Curcuma longa* (11.4%). From the phytochemical screening, alkaloids were found in *Ocimum santrum* and *Zanthoxylum aromaticum*; and glycosides in *Ocimum santrum*, *Colquhounia coccinea* and *Curcuma longa*. Steroids were observed in *Ocimum santrum* and *Zanthoxylum aromaticum* but terpenoids were found in *Rhododendron setosum*, *Colquhounia coccinea* and *Curcuma longa*. Flavonoids were found in *Azadiracta indica* (ethanolic extract), *Colquhounia coccinea* and *Curcuma longa*. Except *Curcuma longa*, all other plants possessed tannins and none of the plants had reducing sugar in their ethanolic extract.

Though the one percent extracts of all the plants showed some degree of antimicrobial activity, it was significant in *Rhododendron setosum*, *Eucalyptus globulus*, *Azadiracta indica* and *Elsholtzia fructicosa*. The extract of *Rhododendron setosum* was effective against *Escherichia coli* (ZI = 2.5 cm). Essential oil of *Eucalyptus globulus* was effective against *Staphylococcus aureus* (ZI = 3.3 cm). Similarly, the extract of *Azadiracta indica* and *Elsholtzia fructicosa* were active against *Klebsiella species* (ZI = 2.5 cm). Zone of inhibition of the individual plant extracts are shown in table 4. Earlier observations done by Srinivasan et al. also showed the antifungal and antibacterial activity of *Azadiracta indica* (Shriniwasan, Nathan, Suresh and Laxamanperumalsamy, 2001).

Table 2. Percentage yield of extracts

Plant	Weight of raw material (gm)	Weight of the extract (gm)	% Yield
Azadiracta indica (ethanolic extract)	120	34.9	29.08
Azadiracta indica (hexane extract)	150	2.6	1.73
Colqhonia coccinea	78	19.3	24.74
Curcuma longa	100	11.4	11.4
Elshotzia fructicosa	64	11.9	18.59
Ocimum sanctum	79.46	14.3	17.99
Rhodendron setosum	118	23.9	20.25
Zanthoxyllum aromatum	100	20.3	20.3

Table 3. Phytochemical constituents of the plant extracts

Plant extracts	Alkaloids	Glycosides	Terpenoids and Steroids	Flavonoids	Tannins	Reducing Sugar
Ocimm santrum	+ve	+ve	+ve (steroid)	-ve	+ve	-ve
Zanthoxylum aromaticum	+ve	-ve	+ve (steroid)	-ve	+ve (catecholic)	-ve
Rhododendron	-ve	-ve	+ve	-ve	+ve	-ve

setosum			(terpenoids)		(catecholic)	
Azadiracta indica (ethanolic extract)	-ve	-ve	-ve	+ve (flavones)	+ve (catecholic)	-ve
Colquhounia coccinea	-ve	+ve	+ve (terpenoid)	+ve (flavonoids)	+ve (catecholic)	-ve
Eltsholtzia fructicosa	-ve	-ve	-ve	-ve	+ve (catecholic)	-ve
Curcuma longa	-ve	+ve	+ve (terpenoid)	+ve (flavonoid)	-ve	-ve

+ve = detected -ve = not detected

Table 4. Zone of inhibition of individual plant extracts

Bacteria	Plants	1% extract solution
Escherichia coli	*Azadiracta indica*	2.3 cm
	Colquhounia coccinea	2.0 cm
	Curcuma longa	1.8 cm
	Elsholtzia fructicosa	2.2 cm
	Eucalyptus globulus (essential oil)	1.8 cm
	Ocimum santrum	2.2 cm
	Rhodendron setosum	2.5 cm
	Zanthoxylum aromatum	2.2 cm
	Gentamicin (0.5%)	3.1 cm
Staphylococcus aureus	*Azadiracta indica*	1.4 cm
	Colquhounia coccinea	2.2 cm
	Curcuma longa	2.4 cm
	Elsholtzia fructicosa	2.1 cm
	Eucalyptus globulus (essential oil)	3.3 cm
	Ocimum santrum	2.1 cm
	Rhodendron setosum	2.4 cm
	Zanthoxylum aromatum	2.6 cm
	Gentamicin (0.5%)	4.7 cm
Klebsiella species	*Azadiracta indica*	2.5 cm
	Colquhounia coccinea	2.2 cm
	Curcuma longa	2.1 cm
	Elsholtzia fructicosa	2.5 cm
	Eucalyptus globulus (essential oil)	2.3 cm
	Ocimum santrum	2.4 cm
	Rhodendron setosum	2.2 cm
	Zanthoxylum aromatum	2.2 cm
	Gentamicin (0.5%)	4.5 cm

CONCLUSIONS

The ethanolic extracts of the studied plants contained many bioactive chemical constituents including alkaloids, glycosides, terpenoids, steroids, flavonoids, and tannins. The extract of *Rhododendron setosum* and the essential oil of *Eucalyptus globulus* were most effective against *Escherichia coli* and *Staphylococcus aureus* respectively but the extracts of *Azadiracta indica* and *Elsholtzia fructicosa* were most effective against *Klebsiella* species.

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