Comparative phytochemical studies of selected medicinal plants in Gwalior (M.P.) region

Sweta Prakash and Meenu Priya Kontu

School of studies in Botany, Jiwaji University, Gwalior (M.P.), 474011

Corresponding author*:* *sweta.shrivastava28@gmail.com*

Abstract

The present study was aimed to evaluate the phytochemical constituents of the leaf extracts of *Barleria prionitis* L., *Cassia tora* (L) Roxb., *Catharanthus roseus* (L.) G. Don. and *Lantana camara* L.Six medicinal plants of ethanobotanical importance belonging to families Acanthaceae, Caesalpiniaceae, Apocynaceae, Verbenaceae, Myrtaceae and Cucurbitaceae were investigated for the presence of alkaloids, flavanoids, flavonols, flavones and flavanols, saponins and steroids in their aqueous extracts. *Barleria prionitis* L., *Cassia tora* (L) Roxb., *Catharanthus roseus* (L.) G. Don., *Lantana camara* L. showed positive tests for alkaloids. All plants gave negative results for cardenolides. *C. tora, C. roseus, L. camara* showed positive results for alkaloids and flavonoides. *B. prionitis*, *C. tora* gave positive tests for flavonols and flavonols and flavononones. *C. roseus*, *L. camara* showed positive tests for alkaloids, flavonoids, flavanols and flavononones.

Key words: Phytochemical, *B. prionitis*, *C. tora, C. roseus, L. camara*, alkaloids, aqueous

Introduction

The World Health Organization has estimated that 80% of the inhabitants of the world rely mainly on traditional plants for their primary health care needs and it may be presumed that a major part of traditional healing involves the use of plant extracts or their active principles. Medicinal plants have been traditionally used for different kinds of infection diseases [Chitravudu *et.al*., 2009] However, several studies have indicated that medicinal plants contain compounds, e.g. peptides, unsaturated fatty acid, aldehydes, flavonoids, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are significant therapeutic application against pathogens, including bacteria, fungus and viruses [Singh et. al. 2010]. These secondary metabolites produced by plants are organic chemicals of

high structural density which play different functions including chemotherapeutic, bactericidal, bacteriostatic and antimicrobial functions [Purohit and Mathur, 1999].

Lantana camara belongs to the family Verbenaceae commonly known as Ghaneri. The leaves of this plant were used as an antitumeral, antibacterial, and antihypertensive agent [Taubi *et. al*. 2007]. In herbal medicine, infusions of the leaves and other plant parts are used as an anti-inflammatory [Odeyapo *et. al*., 1997]. *Barleria prionitis* belongs to family Acanthaceae. Its leaves are used to promote healing of wounds and to relieve joint pains and toothache [Parrotta, 2001]. Because of its antiseptic properties, extracts of the plant are incorporated into herbal cosmetics and hair products to promote skin and scalp health [Prakruti, 2002]. *Catharanthus roseus* belong to family Apocynaceae known as sadabahar. It is cultivated mainly for its alkaloids, which are having anticancer activities [Jaleel *et al*., 2008]. Several research groups have shown that *Catharanthus roseus* has a high potential for many varieties of medicinal properties, such as antibacterial [Carew and Patterson 1970], antifungal [Jaleel *et al*. 2007] and antiviral [Farnsworth *et al*. 1968]. *Cassia tora* belong to family Apocynaceae commonly known as chakoda. The extracts of *C. tora* have been used as a remedy for various skin ailments, rheumatic disease and as laxatives [Hooker, 1879; Kirtikar and Basu, 1975; Jain, 1968]. The extract of *C. tora* leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity [Maitya *et.al*., 1997, 1998].

Methods:

Phytochemical studies (Qualitative chemical analysis):

The plant leaves were air dried in laboratory and ground into uniform powder and stored in container. These plant parts were subjected to qualitative chemical screening for the identification of the various classes of active chemical constituents using standard prescribed methods [Amarsingham *et al.* 1964; Gibbs, 1974].

(i) Test for Alkaloids:

5 gm of dried powder was kept in 50ml of 10% ethanol for 48 hours at room temperature with occasional shaking. The extract was filtered and distilled water vaccum. The dried concentrated extract was acidified with 25ml of 0.1 NH_2SO_4. The acid extract was centrifuged and the clear supernatant was tested with Mayer's, Wagner's and Dragendorff's reagent.

(a) Mayer's Reagent:

1.358 gm of Mercuric Chloride and 5gm of Potassium Iodide dissolved 100ml of water. Both the solutions were mixed and diluted to 100ml with distilled water. To a little of the test filtrate, taken in a watch glass, a few drops of the above reagent were added. Formation of creamy coloured pigment showed presence of alkaloids.

(b) Wagner's reagent:

2 gm of iodine and 6 gm of Potassium Iodide dissolve in 100ml of distilled water. When few drops of this reagent were added to the test filtrate, a brown precipitate was formed indicating the presence of alkaloids.

(c) Dragendorff's reagent:

0.850 gm basic Bismuth nitrate is dissolved in a mixture of 10ml acetic acid and 40 ml distilled water. 8 gm of Potassium Iodide dissolved in 20 ml of distilled water. These stock solutions were mixed together. The above reagent was sprayed on Whatmann filter paper no.1 and the paper was dried. The test filtrates after basification with dilute Ammonia was extracted with chloroform. The chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of an orange red colour on the paper indicated the presence of alkaloids.

(ii) Test for Anthraquinones:

They occur as free anthrons derivatives or as glycosides.

a) The powder was boiled with sulphuric acid for one hour, cooled and filtered. To the filtrate was added Chloroform. The mixture was vigorously shaken and allowed to stand, organic layer gets separated. Ammonia was added to organic layer slowly. Development of red, pink, or violet colour indicates the presence of Anthraquinones. This is to detect glycosides.

b) The powder was extracted with 80% ethanol. The extract was dried. Residue was dried. Residue was dissolved in distilled water, filtered and shaken with benzene in separating funnel 5ml of ammonia added to the benzene layer. Red ammonia layer indicates the presence of Anthraquinones. This is to detect Anthraquinones.

(iii) Test for Cardiac glycosides (Cardenolides):

Fresh tissue was extracted with rectified spirit. To the extract 10% solution of Sodium Hydroxide and 0.3% solution of Nitropruside were added. Appearance of transient pinkish red colouration indicates the presence of Cardenolides.

(iv) Test for Flavonoids:

Different tests were carried out for different types of flavonoids. Tests were carried with dry sample.

(a) Flavonoids (Shinoda test):

To the extract, a piece of Magnesium ribbon and Hydrochloric acid were added. Purple, red, pink or orange colour developed, which confirm flavonoids.

(b) Flavanonols:

If deep colour developed with Shinoda test, then instead of Magnesium ribbon, Zinc powder was added with Hydrochloric acid. Deep magenta colour developed which confirmed flavononols.

(c) Flavonols:

To the extract a pinch of Boric acid and few drops of Acetic acid were added. Bright yellow colour with green fluorescence indicated flavonols.

(d) Flavones and Flavanols:

Firstly extract was dissolved in Sulphuric acid to give yellow solution and the flavanones produced lively orange to crimson colours. To the extract few drops of Sulphuric acid were added and colour was noted. This further confirmed the presence of Flavones, Flavanols and Flavanones.

(e) Rao and Sheshadri test:

To the extract, few drops of Concentrated Nitric acid were added. Brilliant blue colour developed confirmed the presence of phlorglucinol derived flavanones.

(f) Test for Leucoanthocyanin:

In this test, fresh as well as dry sample can be used. 0.5 gm of sample was heated with 2N HCL and given water bath for about 20 minutes. The extract was allowed to cool down at room temperature, filtered and to the filtrate 5ml of Iso-amyl alcohol was added. On the presence of Leucoanthocyanins, the Iso-amyl layer became red. This was noted as '++ve'. When the Iso-amyl layer became pink, the reaction was noted as weak '+ve'; no colour to Iso-amyl layer was noted as '−ve'. In some cases, the Iso-amyl layer became reddish brown. This was denoted as 'thought to be a doubtful case'. The darkening of the colour of solution during boiling (particularly becoming brown) was also noted. The darkening of solution was usually associated with acubin type glycosides in the plant material.

(g) Irridoids / Acubins:

Fresh as well as dry leaves were used for these tests. Leaves were powdered and 5 ml of 1% aqueous hydrochloric acid was added. Extraction was carried out for 6

hours. To the 0.1 ml of extract, 1ml of Trim hill reagent was added. The tube was heated for short time in a flame and colour change was noted. Production of blue, red, violet colour indicates the presence of acubins / irridoids.

Trim Hill reagents: 10 ml of acetic acid + 1 ml 2% aqueous copper sulphate + 0.5 ml concentrated hydrochloric acid**.**

(vi) Test for Simple Phenolics:

Plant powder was extracted with aqueous ethanol overnight. To the extract 1-2 drops of 1% aqueous Ferric chloride was added. Development of specific colours was indicative of the presence of Phenol.

(vii) Test for Saponins:

The powder was extracted with boiling water. After cooling, the extract was shaken vigorously to froath and was then allowed to stand for 10-15 minutes. The persistent froath of 2cm high considered as presence of Saponins.

(viii) Test for Steroids:

2ml of acetic anhydride was added to 0.5 gm ethanolic extract of each sample with 2ml H_2SO_4. The colour changes from violet to blue or green in some samples indicating the presence of steroids.

Table 1: Comparative phytochemical study of *B. prionitis, C. tora, C. roseus, L.camara,* with aqueous extracts

Components	*B. prionitis*	*C. tora*	*C. roseus*	*L. camara*
Alkaloids	-	+	+	+

Anthraquinone	-	+	_	_
Cardenolides	-	_	_	_
Flavonoids	-	+	+	+
Flavononols	_	+	_	+
Flavonols	+	+	_	+
Flavones & Flavanols	+	+	+	+
Flavonones	-	_	_	_
Iridioides	+	_	_	+
Leucoanthocyanin	_	+	_	+
Phenolics	-	+	_	_
Saponins	+	_	+	+
Steroids	-	+	_	+

Results and Discussion:

Aqueous extract of *B. prionitis* showed no inhibition potency. Reports indicate that the antifungal activity is due to presence of different compounds in the extract, including flavonols, flavones, flavanols, irridoids and saponins [Chen *et al*., 1998; Burkill, 1985].This species showed positive tests for alkaloids, flavonols, flavones, flavanols, saponins.

Anthraquinones present in *C. tora* leaves exhibit antimicrobial activity [Rios *et al*., 1987; Diaz *et al*., 1988; Mukherjee *et al*., 1996; Maity, 1999; Goyal *et al*., 2007; Rai and Abdulahi, 1978]. Flavonoids are known to be synthesized by plants in response to microbial infections [Dixon *et al*., 1983]. Acharya and Chaterjee [1975] isolated chrysophanic acid- 9- anthrone, the major antifungal principle in *C. tora*. Leucoanthocyanin have been reported in leaves of *C. tora* [Sofowora, 1982]. They are one of the most powerful bioflavonoids. It improves the strength of the blood vessels including varicose veins. Alkaloids, anthraquinone, flavonoids, flavononols, flavanols, flavonols, flavones, leucoanthocyanin, saponin and steroids were found to be present in its leaf powder.

About 150 alkaloids have now been isolated from *C. roseus* some important are ajmalicine, lochnerine, serpentine and tetrahydroalstonine; occur in various genera of this family. Presences of alkaloids,

flavonoids, flavones, flavanols, favonols, saponins and phenolics have been found in various concentrations in the plants of this family [Mustafa and Verpoorte 2007]. The species exhibited positive test for alkaloids, flavonols, flavanols, flavones, irridoides, saponins, phenolics and steroids

The medicinal properties of this species have attributed due to presence of an antimicrobial flavonoid 'umuhengerin' isolated from the leaves of *L. camara* have also been reported to contain flavononols, flavonols, flavonoids and flavones [Anonymous, 2005; Barre *et al*., 1997; Majekodunmi *et al*. 2002 and Ghisalberti, 2000]. Irridoid glycosides have also been reported from this species. They appear to be the characteristic features of the family. Various compounds such as alkaloids, flavonols, flavones, flavanols, flavonoids, leucoanthocyanin, steroids, saponin, irridoides were present in it.

1) Anthraquinones, flavonoids, saponins and steroids were noticed to be present *L. camara*.
2) Alkaloids, anthraquinone were observed in *B. prionitis* leaves.
3) Irridoides, leucoanthocyanin were present in *B. prionitis, C. tora, L. camara*.

Conclusions :

The research work showed the presence of various phytochemical constituents in *C. tora, L. camara* in aqueous extract. The selected plants are the source of secondary metabolites. *C. tora*, *L. camara* plant shows the variation in its constituents which are useful in the manufacturing of drugs. These types of studies have vital role because of the commercial and research interest. Further research is necessary to isolate and determine the identity of the active compound.

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