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PHYTOCHEMICAL ANALYSIS OF THREE ENDANGERED PLANTS (*COSTUS SPECIOUS*, *GLORIOSSA SUPERBA* LINN AND *RAUVOLFIA SERPENTINE* (LINN) BENTH) FROM KANKER DISTRICT OF CHHATTISGARH, INDIA

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ABSTRACT
Chhattisgarh, the herbal state houses with rich and unique biodiversity of medicinal plants which are widely used by traditional healers of the state for treatment of various disease and extensively exploited for commercial purpose which leads to serious threat. Several plants have been studied and listed as rare and endangered which need to be conserved. We selected three such plants viz. *Costus specious, Gloriossa superba* Linn and *Rauvolfia serpentine (*Linn*)* Benth.*,* from Kanker district of Chhattisgarh for their phytochemical analysis by chemical screening and Thin layer chromatography. On primary analysis of the methanolic and ethanolic extract of rhizome of *C. specious* and *G. superba* and root and leaves of *R. serpentine (*Linn*)* Benth. we found the presence of phytocompounds like saponin, tannins, alkaloids, terpenoids, flavonoids and glycosides in them. This phytocompounds were further separated by TLC using petroleum ether: methanol: benzene (8:1:1.5) as mobile phase in silica gel coated glass plates. Maximum 9 bands were observed in methanolic extract of rhizome of *C. specious* with Rf value between 0.05 – 0.97 and 6 bands in methanolic extract of rhizome of *G. superba* with Rf value between 0.02 – 0.94.Similarily in ethanolic and methanolic extract of leaves of *R. serpentine (*Linn*)* Benth. Maximum 13 bands were observed with Rf value between 0.05 – 0.96 and 0.04 – 0.98 respectively whereas ethanolic extract of root showed maximum 11 bands with Rf value between 0.04 – 0.98 in comparison with chloroform and petroleum ether extracts. This result can further help in development of new drugs for diseases like cancer and hypertension based on their traditional uses.

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases (Parekh and Chanda, 2007).

Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Tiwari *et al*., 2011).

The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dandapat *et al*., 2013; Kullu *et al*., 2013; Kumar *et al*., 2013; Kumar *et al*., 2013a; Mahato *et al*., 2013; Tabassum *et al*., 2013; Toppo *et al*., 2013; Sahu *et al*., 2013).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total are used appropriately. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects and herbivores. Furthermore, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines) and flavor (capsacin). However, several of these molecules possess medicinal properties (Britto *et al*., 2011).

The herbal state Chhattisgarh in the Deccan biogeographic area, which houses rich and unique biological diversity, has a rich source of many endemic plants with medicinal importance. Plants belonging to more than 911 genera and 196 families are included in the inventory of medicinal plants of the state. Out of which 90% are naturally growing and 10% are cultivated by farmers. But with rise in demand of the raw herbs and herbal product the pressure on supply side has increased and destructive harvesting has been started, result of which there are number of plants became threatened and endangered (CSMPB, 2006). Nation Botanical Research Institute (NBRI), Lucknow had identified and listed 45 species as endangered taxa of the state which need to be conserved. (CSMPB, 2006).

Thus in present study we have selected three endangered species of the Chhattisgarh state viz. *Costus specious, Gloriosa superba* Linn and *Rauvolfia serpentine (*Linn*)* Benth. for primary screening of secondary metabolites present in them and their

Table 1: Showing presence of different phytocompounds in ethanolic extract of different parts of *R.serpentine, G.superba* and *C.specious*

S.No.	Phytocompounds	*R. serpentine* Leaves	*R. serpentine* Root	*G. superb* Rhizome	*C. specious* Rhizome
1.	Tannins	+	-	+	-
2.	Flavonoids	-	+	+	+
3.	Saponins	+	+	-	+
4.	Glycosides	+	+	+	+
5.	Steroids	+	+	+	+
6.	Alkaloids	+	+	+	+

+ = Present; - = Absent

Table 2: Showing presence of different phytocompounds in methanolic extract of different parts of *R.serpentine, G.superba* and *C.specious*

S.No.	Phytocompounds	*R. serpentine* Leaves	*R. serpentine* Root	*G. superba* Rhizome	*C. specious* Rhizome
1.	Tannins	+	-	-	-
2.	Flavonoids	-	+	+	-
3.	Saponins	+	+	+	+
4.	Glycosides	+	+	+	+
5.	Steroids	+	+	+	+
6.	Alkaloids	-	-	+	+

+ = Present; - = Absent

Table 3: Showing RF value in TLC chromatogram of different extracts of rhizome of *G. superba*

No. of bands	Ethanol extract	Methanol extract	Chloroform extract	Petroleum ether extract
Band 1	0.04	0.05	0.03	0.00
Band 2	0.09	0.08	0.11	0.09
Band 3	0.23	0.13	0.55	0.84
Band 4	0.99	0.19	0.99	0.89
Band 5	-	0.27	-	0.94
Band 6	-	0.41	-	0.97
Band 7	-	0.58	-	-
Band 8	-	0.72	-	-
Band 9	-	0.97	-	-

Table 4: Showing RF value in TLC chromatogram of different extracts of rhizome of *C. specious*

No. of bands	Ethanol extract	Methanol extract	Chloroform extract	Petroleum ether extract
Band 1	0.91	0.02	0.10	0.93
Band 2	0.94	0.12	0.91	0.96
Band 3	-	0.19	0.98	-
Band 4	-	0.86	-	-
Band 5	-	0.92	-	-
Band 6	-	0.94	-	-

TLC profiling.

MATERIALS AND METHODS

The three plant species selected for the study viz. *Costus specious* (Fig. 2), *Gloriossa superba* Linn (Fig. 3) and *Rauvolfia serpentine (*Linn*)* Benth (Fig. 4) were collected from Kanker (20º6'-20º24'N; 80º48'- 81º48' E) district of Chhattisgarh, India (Fig. 1) in the month of June, 2012 and were then compared with herbaria of CSMPB and identified by Botany department of the college. The collected plants were then washed and surface sterilized and dried in shade. After drying extracts were prepared form different part of plant viz. rhizome of *C. specious* and *G. superba* Linn and leaf and root of *R. serpentine* (Linn*)* Benth in Soxh let extractor using 4 different solvents ethanol, methanol, chloroform and petroleum ether.

From the methanol and ethanol primary screening for the presence of various secondary metabolites like steroids, tannins, saponins, alkaloids, flavonoids and glycosides (Trease and Evans, 1989) was performed followed by thin layer chromatography of all four extracts using silica gel coated glass plate as stationary phase and solvent system containing petroleum ether: methanol: benzene (8:1:1.5) as mobile phase. The plates were then exposed in UV light to observe the migration pattern of bands and Retention factor (Rf value) of band were calculated using the formula,

Rf = Distance Travelled by substance/ Distance Travelled by solvent

RESULTS AND DISCUSSION

Primary screening of ethanolic and methanolic extract of plant parts showed the presence of tannins, flavonoids, glycosides, steroid, and alkaloids in ethanolic extract of rhizome of *G. superba* whereas methanolic extract showed presence of all secondary metabolites studied except tannin. Ethanolic extract of rhizome of *C. specious* also showed the presence of flavonoids, saponins, glycosides, steroid, and alkaloids except tannins but in its methanolic extract tannins and flavonoids were absent. In ethanolic extract of leaves of *R. serpentine,* flavonoids was absent and in root extract tannins was absent, other phytochemicals were found present in both extract of the plant (Table 1). In methanolic extract of leaves of *R. serpentine* tannins, saponins, glycosides and steroids were present and in root flavonoids, saponins, glycosides and steroids were present (Table 2).

TLC profiling of extract of rhizome of *G. superba* in all four solvents viz. ethanol, methanol, chloroform and petroleum ether was performed among which it showed maximum 9 bands in methanolic extract with Rf value ranging from 0.05 to 0.97 followed by petroleum ether extract with 6 bands with Rf value in the range of 0.00 to 0.97 whereas ethanol and chloroform extract showed 4 bands each with maximum Rf

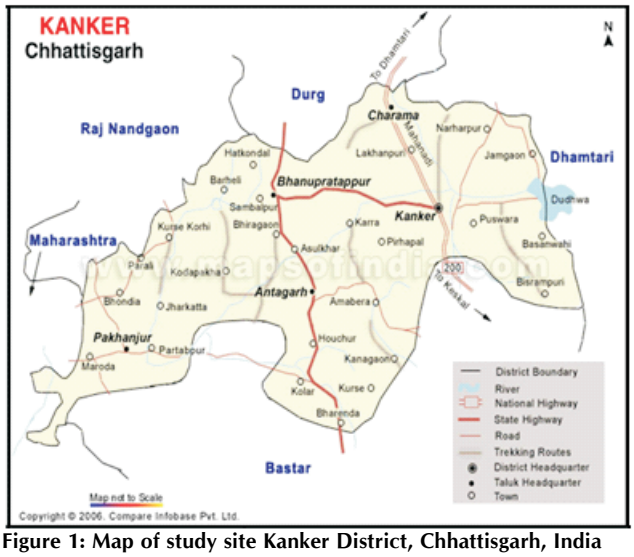
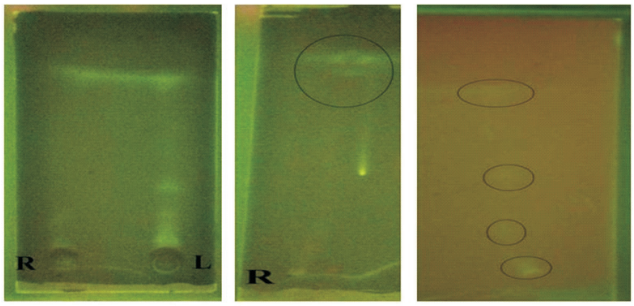


Figure 1: Map of study site Kanker District, Chhattisgarh, India

Figure 2: Plant of *Costus specious*

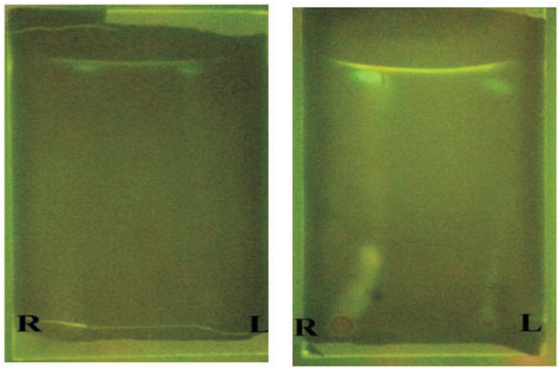
Figure 3: Plant of *Gloriosa superba*

Figure 4: Plant of *Rauvolfia serpentine*



(A) (B) (C)

Figure 5: TLC chromatogram of rhizome of *G. seperba* (A) R = Methanolic extract, L = Ethanolic extract; (B) R = = PE extract; (C) Chloroform extract



(A) (B)

Figure 6: TLC chromatogram of rhizome of *C. specious* (A) R = Ethanolic extract, L = Methanolic extract; (B) R = Chloroform extract, L = PE extract

value of 0.99 (Table 3, Fig. 5). Similarly methanolic extract of rhizome of *C. specious* showed maximum 6 bands with Rf value ranging between 0.02 to 0.94 followed by 3 bands with Rf value in range 0.10 to 0.98 in chloroform extract, and 2 bands each in ethanol and petroleum ether extract of rhizome (Table 4, Fig. 6). In *R.serpentine* leaves 13 bands each was observed in methanolic, ethanolic and chloroform extract with Rf value ranging between 0.05 to 0.96 in ethanolic extract, 0.04 to 0.98 in methanolic extract and 0.09 to 0.97 in chloroform extract whereas only 4 bands in range 0.60 to 0.94 was observed in petroleum ether extract (Table 5, Fig. 7). Ethanolic extract of root of *R.serpentine* showed 11 bands with Rf value between 0.04 to 0.98 followed by methanolic extract with 9 bands with Rf value between 0.05 to 0.89, petroleum ether and chloroform does not have any band on TLC of root extract (Table 6, Fig.8).

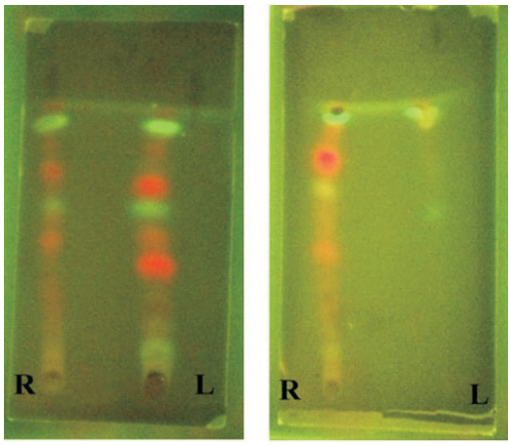
Crude preparations of different plants are used by traditional healers of the state for treatment of various diseases. These crude preparations contain a number of phytochemicals like alkaloids, saponins, tannins, terpenoids etc. which is responsible for the potential medicinal values of the plants. Alkaloids are reported to having antipyretic, antitumor, muscle relaxant, anti-cough activity (Meyers, 2001; Hesse, 2002). Flavonoids found in plants are having anti microbial and

Table 5: Showing RF value in TLC chromatogram of different extracts of leaves of *R. serpentine*

No. of bands	Ethanol extract	Methanol extract	Chloroform extract	Petroleum ether extract
Band 1	0.05	0.04	0.09	0.60
Band 2	0.12	0.11	0.15	0.81
Band 3	0.14	0.17	0.27	0.90
Band 4	0.20	0.25	0.35	0.94
Band 5	0.27	0.33	0.49	-
Band 6	0.41	0.49	0.61	-
Band 7	0.52	0.50	0.68	-
Band 8	0.62	0.62	0.78	-
Band 9	0.69	0.74	0.89	-
Band 10	0.82	0.84	0.93	-
Band 11	0.88	0.93	0.95	-
Band 12	0.91	0.97	0.96	-
Band 13	0.96	0.98	0.97	-

Table 6: Showing RF value in TLC chromatogram of different extracts of roots of *R. serpentine*

No. of bands	Ethanol	Methanol extract	Chloroform extract	Petroleum ether extract
Band 1	0.04	0.05	-	-
Band 2	0.05	0.07	-	-
Band 3	0.14	0.17	-	-
Band 4	0.26	0.27	-	-
Band 5	0.42	0.54	-	-
Band 6	0.51	0.64	-	-
Band 7	0.63	0.72	-	-
Band 8	0.68	0.84	-	-
Band 9	0.72	0.89	-	-
Band 10	0.82	-	-	-
Band 11	0.98	-	-	-



(A) (B)

Figure 7: TLC chromatogram of leaves of *R. serpentine* (A) R = Methanolic extract, L = Ethanolic extract; (B) R = = Chloroform extract, L = PE extract

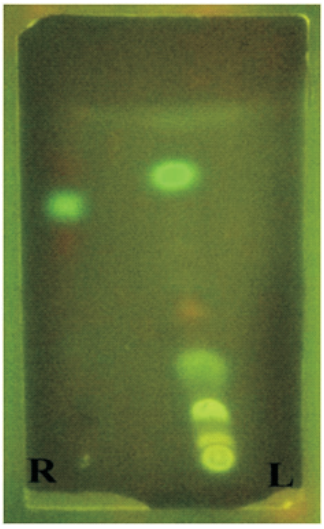


Figure 8: TLC chromatogram of roots of *R. serpentine,* R = Methanolic extract, L = Ethanolic extract.

antioxidant activity (Honda *et al.,* 1984; Lin *et al.,* 2002) whereas tannins are used for wound healing and as astringent agent, and are reported to be having antiviral (Rangari, 2007), antioxidant (Amarowicz *et al.,* 2004), antimicrobial (Ho *et al.,* 2001), gastroprotective and anti-ulcerogenic activities (Ramirez and Roa, 2003). Saponins are also known to possess anti-inflammatory (Balandrin, 1996), hypocholesterolemic (Oakenfull, 1996) and immune-stimulating (Klausner, 1988) properties. Similarly terpenoids are also having various pharmacological activities like anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer (Mahato and Sen, 1997; Kaneda *et al.,* 1992; Rasadah *et al.,* 2004 Muhammad *et al.,* 2000). We have also reported the presence of these groups of phytocompounds in different parts of *G. superba, C.specious* and *R. serpentine* studied which is responsible for pharmaceutical and nutraceutical values of these plants. *C. specious* is having alkaloids, tannins, saponins etc. in its rhizome which is used as caridiotonic and as CNS depressant (Khare, 2007; Bhattacharya *et al.*, 1973), it is also having anti-inflammatory and antiarthritic effect, antifungal (Dubey *et al.*, 2000) activities. Rhizome of *G. superba* also contain flavonoids, saponins, steroid and alkaloids which is used as antidote against snake-bite (Asolkar *et al.*,1992) and insect bite (Chitra and Rajamani 2009) and for the treatment of bruises and sprains, colic, chronic ulcers, haemorrhoids, cancer, impotence, and leprosy and also for including labour pains and abortions (Kala, 2011). *G. superba* also used in wounds, skin related problems, fever, Inflammation, piles, blood disorders, uterine contractions (Haroon *et al.*, 2008; Srivastava and Chandra, 1977). Roots of *R. serpentine* is used as remedy for high blood pressure, insomnia, anxiety, excitement (Dey and De, 2011), in disorders of gastro intestinal tract viz. diarrhea, dysentery and cholera and colic and even for cancers (Deshmukh *et al.*, 2012). Leaves are used in removal of opacities of cornea and as a fever relieving medicine. (Deshmukh *et al.*, 2012) Roots and leaves of *R. serpentine* are also possessed tannins, flavonoids, alkaloids etc which is responsible of its wide medical application. Thus, present finding provide scientific validation to knowledge of our traditional healers. The work can be further extended for isolation of specific compounds with pharmaceutical importance which can be helpful for pharmaceutical industries for the development of new drugs for disease like cancer and hypertension etc. Industrial application of these plants will lead to their commercial cultivation which will also be helpful in their conservation and making a balance in the nature.

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