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Original Research Article

Isolation and purification of alkaloids from medicinal plants by HPLC

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Keywords

TLC; UV Light; Alkaloids; photo-chemical medicinal plants; HPLC; Assay.

A B S T R A C T

Methanolic extracts of 28 medicinal plants were evaluated for the presence of secondary metabolites by TLC. Among the 28 methanolic extracts of medicinal plants Gujpatta (*Abrus precabrius*), Sadapatta, shankhapushpi *(Canscora decussate)* and makka *(Zea nays)* were concentrated and use for purification of secondary metabolites by HPLC. HPLC profile of sample *Abrus precabrius* revealed 3 major peaks, as item 2, item 4, item 9, with retention time 3.203 min, 5.330 min, 7.760 min respectively and their respective constrations are 0.000 ug/ml, 6.116 ug/ml, 19.557 ug/ml. HPLC profile of sample Sadapatta revealed 3 major peaks, as item 2, item 7, item 9, with retention time 3.245 min, 6.104 min, 8.052 min respectively and their respective constrations are 0.502 ug/ml, 26.516 ug/ml, 12.835 ug/ml. HPLC profile of sample *canscora decussate* revealed 8 major peaks, as item 1, item 2, item 3, .item 4 item 5, item 6 item 7, item 8, With retention time 0.692min, 0.850 min, 3.255 min, 3.747 min, 3.830 min, 5.292 min, 6.017 min, 8.372 min respectively and their respective constrations are 0.006 ug/ml, 0.009 ug/ml, 0.247 ug/ml, 0.524 ug/ml, 0.290 ug/ml, 5.212 ug/ml, 35.521 ug/ml, 24.151 ug/ml. HPLC profile of sample *Zea nays* revealed 3 major peaks, as item 4, item 7, item 9, With retention time 5.323 min, 5.858 min, 7.820 min respectively and their respective constrations are 16.701 ug/ml , 31.048ug/ml. This study given a scientific basis to plants already used for traditional purposes and also prove new antimicrobial constituent from randomly selected plants whose anti-infective properties have not been evaluated.

Introduction

Medicinal plants are still major part of traditional medicinal system in developing countries many infection disease are known to be treated with herbal remedies throughout the history of mankind even today plant material continue to play a major role in primary health care as therapeutic remedies in many developing countries (Sukarya *et al.,* 2009) Medicinal plants which from the backbone of traditional medicine have in the last few decades been the subject of very instance

pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plant as potential source of new compounds of therapeutic value and as source of new compounds in drug development. in many parts of the words medicinal plants are used for antimicrobial centrifugal and antiviral activities as plant derived drugs serve as a prototype to develop more effective and less toxic medicine. Tribal medicine has not been studied extensively (Zakaria, 1989) The alkaloids are one of the most divorcees groups of secondary metabolites found in living organisms and have an array of structure type, biosynthetic pathway, and pharmacological activities.(Margaret f. Robert and Michael wink) Although alkaloids have been traditionally isolated from plants an increasing number are to be found in animal's insects and marine invertebrates and microorganisms. Many alkaloids have been used for hundreds of years in medicine and same are still prominent drugs today, hence this group of compounds has had great prominence in many fields of scientific endeavor and continues to be of great interest today (Margaret f. Robert and Michael wink) Plant have a limit less ability to synthesize aromatic substance mainly secondary metabolites of which at least 12,000 have been isolated a number estimated to be less than 10% of the total (Mallkharjuna *et al.,* 2009) The synthesized aromatic substances (metabolite) are used by plants as defensive molecule against predation by microorganism insects and herbivores. However some of which may involve in plant odor (terpenoids), pigmentation (tannins) and flavor (capsaicin) (Mallikhaarjuna et at 2009) Using plant for medicinal purposes is important part of the culture and the tradition in Africa. Thus up to 80% of the population depends directly on the traditional medicine for the primary health care. (Kirby, 1996) Research focused on malaria leads to the identification of alkaloids, principally cryptolepine, the major alkaloid of the plant as its antibacterial agent (karou et at 2003) We found that polyphenol extract of the plant had a weak antioxidant activity through in vitro free radicals scavenging assays on the other hand the extract was very active on pathogen bacteria and this activity may be influenced by the polymerizations size of the phenolic compounds. (Karaou *et al*., 2005) The effects of plant extracts an microorganism has been studied by a very large number of researches in different part of the word. (Mahesh and Satish 2008,) The specific function many photochemical is still unclear how ere a considerable number of studies have shown that they are involved in the interaction of plants /pests /disease. (Cooper *et al*., 2006) Scientist from divergent fields are investigating plants with a new age for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source o as well as specific activity. (Nair and chanda 2007) There is a need to develop alternative antimicrobial drugs for the treatment of infections diseases from medicinal plants. Several screening studies have been carried out in different part of the antimicrobial activity of different herbal extract in different regions of the worlds. (Nair *et al*., 2005).

HPLC is highly sensitive method for detection, identification and quantification of any chemical in a particular samples using UV and visible absorbance(Hanachi and Golkho, 2009) By comparing with the retention time of the standards , phenolic compounds can be identified(Sarma *et al*., 2002).

Materials and Methods

Collection and identification of plants

The leaves, bark, roots and seeds of 28 medicinal plants which are used in this study are in powdered form collected from the "Davasaaj Ayurvedic store" a medicinal plant seller from local market in Aurangabad, Maharashtra. The plants were identified with the help of botany department of Vinayakrao pail mahavidyalaya, Vaijapur, Dist. Aurangabad. (Table No 1).

Solvent extraction

All 28 medicinal plants were collected in a powders form. This was prepared by dissolving 1gm powder sample in 10ml of methanol in a test tube (Fig.No. 1). These were kept for 24hrs at room temperature for solvent extraction. The supernatant were collected and stored for further studies.

Separation, Identification and Confirmations of Secondary metabolites

Separations of alkaloids from all the 28 medicinal plant samples were done by the TLC method. The solvent system used for TLC is chloroform: methanol (15:1). Secondary metabolites were identified by using UV light (UV transilluminator) Under UV light secondary metabolites gives violet colure florescence.

Purification & Qualitative and quantitative analysis of secondary metabolites by HPLC system

1gm of sample kept overnight for extraction in HPLC grade methanol. This extracts were sonicated for 20 min in sonicator 20 ul from sonicated extracts was passed through 0.45 mm filter. Filtrate was used for HPLC analysis. Qualitative and quantitative HPLC analysis of the sample was performed according to the method of sarma et.al, (2002). The HPLC system (cyber lab chromo HPLC) was used. The software package used for analyzing results was cyber lab chromo HPLC control and sampling.Chromatographic analysis was carried out using a c-8 column at temperature: ambient. Running conditions included: injection volume 20ul; mobile phase: methanol: water (60:40), flow rate 0.500 ml\min; and detection at 205nm. Samples were filtered through an ultra membrane filter (pore size 0.45um) prior to injection in the sample loop.

Data analysis

Retention time and concentration of phenolic acid was identified by using HPLC software (cyber lab chromo HPLC control and sampling).

Results and Discussion

TLC analysis of medicinal plants

Among 28 medicinal plants only 18 plants gives positive results as shown in (Table no.3). Gujpatta, Sadapatta, Shankhapushpi and makka, were given instance bands of secondary metabolites (bands are shown in fig.no.3 and table no.4)

HPLC analysis of samples revealed that highest peaks were present in makka, shankhapusphi, Sadapatta, Gujpatta. Fig.no.4 HPLC profile of *Zea maize* reveled 3 major peaks as item 4, item 7, item 9 with retention time 5.323 min, 5.858 min, 7.820 min respectively and their respective concentrations are 16.701 ug/ml , 31.048ug/ml respectively.Fig.no.5

Fig.1 methanolic extractions

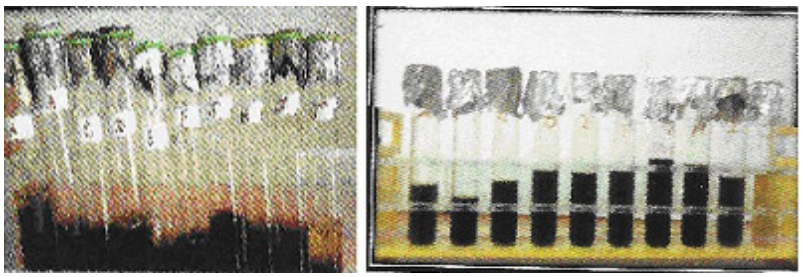
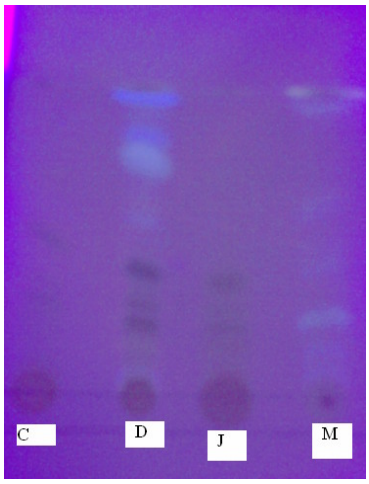


Fig.3 showing TLC result of the band of interest of compound no: - Gujpatta (*Abrus precabrius*), Sadapatta, shankhapushpi *(canscora decussate)* and makka *(Zea nays)*



Sample no:4 (D) Sadapatta gives 5 bands from which band no:2 and 5 are the instanced bands (counting of bands from the bottom to top of TLC).
Sample no: 13 (M) makka gives 6 bands from which band no: 1 and 5 are the instanced ones.
Also sample no: 3 (C) Gujpatta and sample no.10 (J) shankhapusphi both gives one instanced each.

Fig.4 HPLC chromatogram of sample (M) Makka *(Zea maizs)*

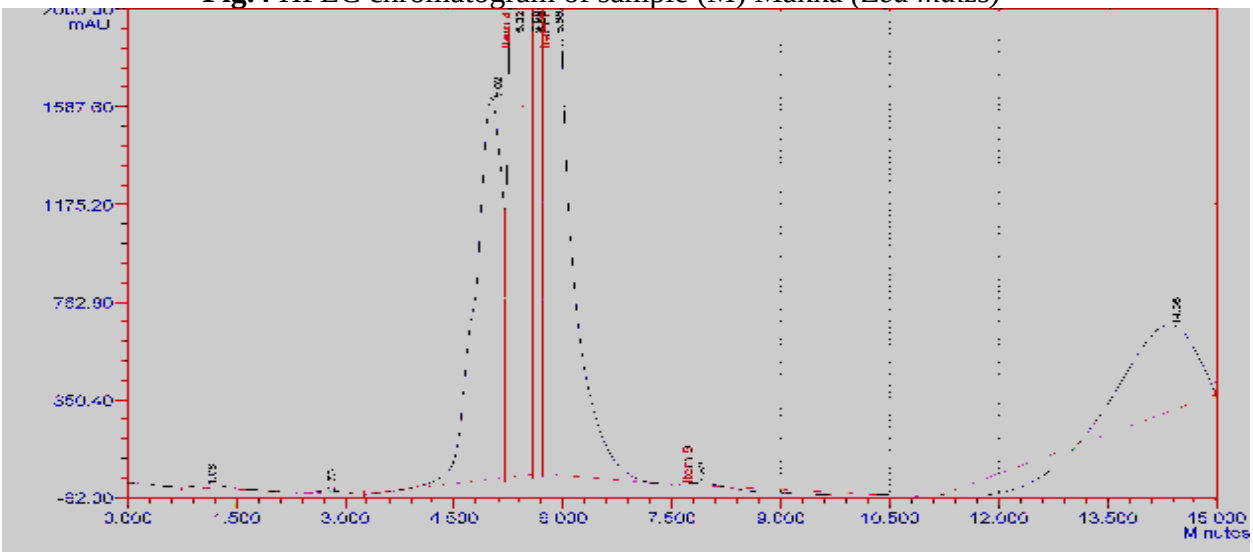


Table.1 The list of all 28 medicinal plants with there scientific name

Sr.no	**Sample name**	**Scientific name**
1	Rjhance	*Not Known*
2	Bhutakes	*Not Known*
3	Gujpatta	*Abrus precabrius*
4	Sadapatta	*Not Known*
5	Pudina	*Menthal spicata*
6	Naypatta	*Not Known*
7	Ratanjot	*Jatropha curcus*
8	Bunphasha	*Not Known*
9	Gulebanphasha	*Not Known*
10	Shankhapusphi	*Canscora decussate*
11	Sanna	*Cassia*
12	Brahmi	*Herpestis monniera*
13	Makka	*Zea mays*
14	Aphumari	*Opium poppy*
15	Mehandi	*Lawsonia alba*
16	Chirayta	*Stwertia chirayta*
17	Tulsi	*Ocimum sanctum*
18	Ashwagandha	*Withania seminifera*
19	Bhuitarwat	*Cassia auricalata*
20	Nirgudipatta	*Vitex negundo*
21	Hirda	*Terminalia chebula*
22	Khair	*Acacia chomdra*
23	Triphal	*Not Known*
24	Baheda	*Terminalia sellirca*
25	Jamun	*Syzygium culmini*
26	Awala	*Phyllumthus embilu*
27	Babul	*Acacia nilotical*
28	Dalimb	*Punico gronataty*

Table.3 Confirmation test result of alkaloids

Sr.No	Sample Name	Test	No. of Bands
1	Rjhance	+	1
2	Bhutakes	+	3
3	*Abrus precabrius*	+	1
4	Sadapatta	+	5
5	*Menthal spicata*	+	1
6	Naypatta	+	2
7	*Jatropha curcus*	+	1
8	Bunphasha	+	3
9	Gulebanphasha	-	0
10	*Canscora decussate*	+	1
11	*Cassia*	+	1
12	*Herpestis monniera*	-	0
13	*Zea mays*	+	6
14	*Opium poppy*	-	0
15	*Lawsonia alba*	-	0
16	*Stwertia chirayta*	+	1
17	*Ocimum sanctum*	-	0
18	*Withania seminifera*	+	2
19	*Cassia auricalata*	+	2
20	*Vitex negundo*	+	1
21	*Terminalia chebula*	+	2
22	*Acacia chomdra*	-	0
23	Triphal	-	0
24	*Terminalia sellirca*	-	0
25	*Syzygium culmini*	+	4
26	*Phyllumthus embilu*	+	0
27	*Acacia nilotical*	+	2
28	*Punico gronataty*	-	0

Table.4 showing bands of interest (interest/dark band)

Sr.no.	Sample name	**No. of Bands**	**Band of interest**	**Light band**
1	Rjhance	1		1
2	Bhutakes	3		3
3	*Abrus precabrius*	1	1	1
4	Sadapatta	5	2,5	1,3,4
5	*Menthal spicata*	1		1
6	Naypatta	2		2
7	*Jatropha curcus*	1		1
8	Bunphasha	3		3
9	Gulebanphasha	0		0
10	*Canscora decussate*	1	1	1
11	*Cassia*	1		1
12	*Herpestis monniera*	0		0
13	*Zea mays*	6	1,5	2,3,4,6
14	*Opium poppy*	0		0
15	*Lawsonia alba*	0		0
16	*Stwertia chirayta*	1		1
17	*Ocimum sanctum*	0		0
18	*Withania seminifera*	2		2
19	*Cassia auricalata*	2		2
20	*Vitex negundo*	1		1
21	*Terminalia chebula*	2		2
22	*Acacia chomdra*	0		0
23	Triphal	0		0
24	*Terminalia sellirca*	0		0
25	*Syzygium culmini*	4		4
26	*Phyllumthus embilu*	0		0
27	*Acacia nilotical*	2		0
28	*Punico gronataty*	0		2

Fig.5 HPLC chromatogram of Sample (D) Sadapatta

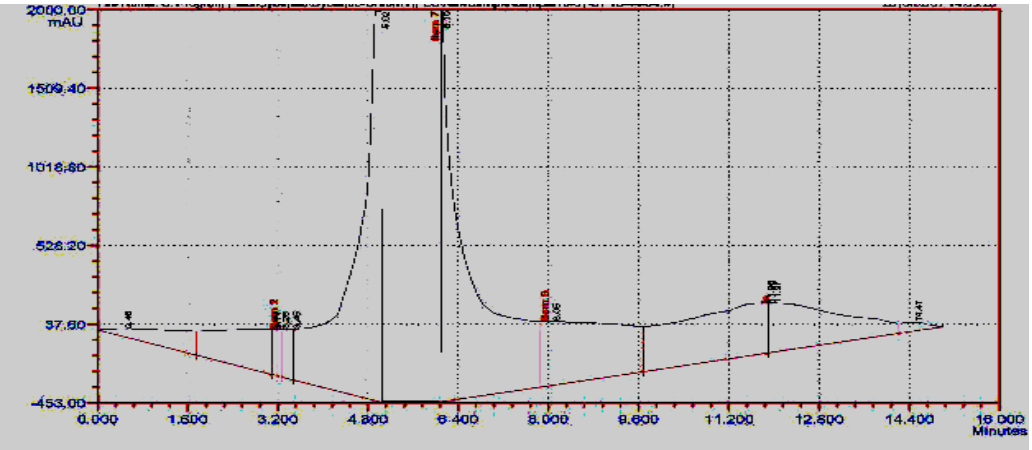


Fig.6 HPLC chromatogram of Sample Gujpatta (*Abrus precabrius*),

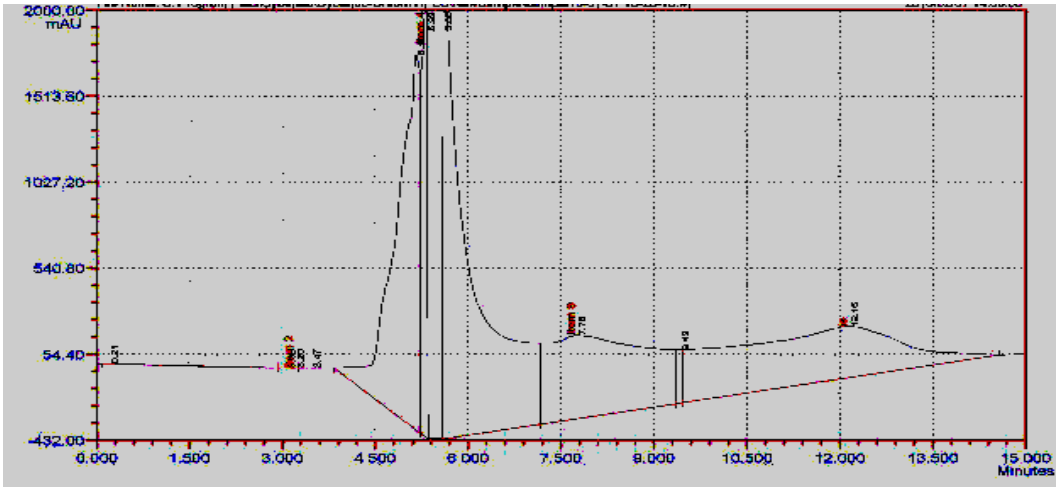
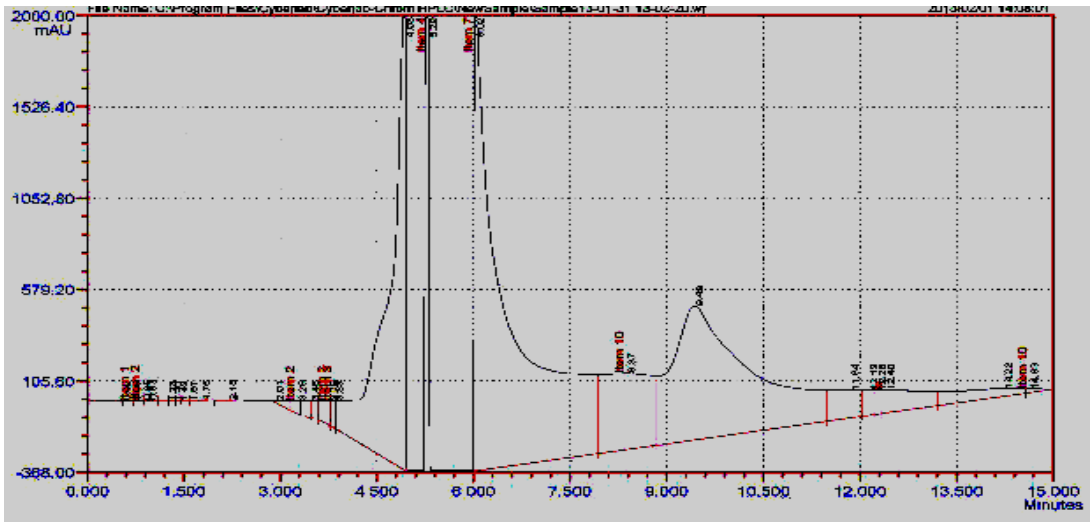


Fig.7 HPLC chromatogram of Sample J C Shankhapushpi *(canscora decussate).*



HPLC chromatogram of Sadapatta, HPLC profile of sadapatta, reveled 3 major peaks as item 2, item 7, item 9 with retention time 3.245 min, 6.104 min, 8.052 min respectively and their respective constrations are 0.502 ug/ml , 26.516 ug/ml,12.835 ug/ml. Fig.no.6 HPLC profile of *Abrus precabrius*, reveled 3 major peaks as item 2, item 4, item 9 with retention time 3.203 min, 5.330 min, 7.760 min respectively and their respective concentrations are 0.000 ug/ml , 6.116 ug/ml,19.557 ug/ml. Fig.no.7 HPLC profile of *canscora decussate* reveled 8 major peaks as item 1, item 2, item 3,.item4,item5, item 6,item 7, item 8 with retention time 0.692min, 0.850 min, 3.255 min,3.747 min, 3.830 min, 5.292 min , 6.017 min, 8.372 min respectively and their respective concentrations are 0.006 ug/ml , 0.009 ug/ml,0.247 ug/ml, 0.524 ug/ml, 0.290 ug/ml, 5.212 ug/ml, 35.521 ug/ml, 24.151 ug/ml.

The role of phenolic as secondary metabolites in living organism is to induce resistance among the both for existence. The phenolic acids are secondary metabolites extensively spreads through the plant kingdom phenolic compounds confer unique taste flavor and health promoting properties found in vegetables and fruits. In recent years the importance of antioxidant activity of phenolic compound and their potential uses. In processed foods as a natural antioxidant compound has reached a new level. Phenolic acid compound and function have been the subjected of great no of agriculture, biological chemical and medical studies. Like phenolic acid flavonids are categorized as secondary metabolites flavonids are also well known for their antioxidant activity. Antioxidant are specific compounds that project human animal and plant cell against the damaging effects of the radicals (ROS) recently phenolic and flavonoids have been considered as great antioxidants and proved to be more effective than vit.C,E & A.

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