IJPSR (2013), Vol. 4, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 December, 2012; received in revised form, 16 January, 2013; accepted, 18 March, 2013

PHYTOCHEMICALS INVESTIGATION AND TLC PROFILING OF *CYAMOPSIS TETRAGONOLOBA* L. SEEDS (FABACEAE) - PEA FAMILY

Sunil H. Ganatra*, Archana M. Ramteke, Shweta P. Durge and S.U. Patil

Department of Chemistry, Government Institute of Science, Nagpur (Affiliated to Rashtrasant Tukdoji Maharaj Nagpur University), Nagpur, Maharashtra, India

Keywords:
TLC, Terpenoid, Saponin, Phenol, Solvent Extract, Plant Extraction

Correspondence to Author:

Dr. S. H. Ganatra

Associate Professor, Department of Chemistry, Institute of Science, Nagpur R. T. Road, Civil Lines, Nagpur – 440001, Maharashtra, India

E-mail: sunilganatra@gmail.com

ABSTRACT: *Cyamopsis tetragonoloba* L. is belongs to the family *Fabaceae.* It is used as herbal and as vegetable from ancient times. It is abundant in tropical region of Africa and Asia. Preliminary phytochemicals investigations have been carried out on the seed extract using n-Hexane, Ethyl Acetate, Acetone, Ethanol and Methanol solvents. Qualitative phytochemicals analysis reflects the presence of Phenol, Quinone, Steroid, Flavanoids and Terpenoid in the plant extract. TLC profiling of seed extracts give an idea about the presence of various phytochemicals. Different R_f (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals.

INTRODUCTION: *Cyamopsis tetragonoloba* L. (syn. *C. psoraloides*) or cluster bean (also called as Guar, Guwar and Guvar bean), is a drought-tolerant summer annual legume and it is cultivated as a feed crop for human and livestock consumption. *Cyamopsis tetragonoloba L.* plant's growth characteristics make it beneficial for arid production areas. Guar is drought resistant, does not require irrigation, and adapts to various soil types.

Cyamopsis tetragonoloba L. belongs to the family *Leguminaceae*, it is grown in tropical Africa and Asia. *C. tetragonoloba* bean is commercially grown for its seeds as a source of natural polysaccharide (galactomannan), commercially known as guar gum [1, 2].

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
www.ijpsr.com

The seed is hard, flinty, flattened, ovoid, measures about 5 mm long, white, grey or black. Seedling is with epigeal germination. Guar gum has a number of uses in food [3] and other industries, such as paper, textiles, oil well drilling and pharmaceuticals [4]. *C. tetragonoloba* L. is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia [5]. In addition, *Cyamopsis tetragonoloba* L. beans are potentially high sources of additional phytochemicals [6].

For the pharmacological as well as pathological discovery [7] of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemicals screening of plant extracts. The aim of this study is to optimize extraction methods in order to maximize the recovery of secondary metabolites in the crude extracts of *C. Tetragonoloba* seeds. This was accomplished by examining the influence of different extraction solvents on the presence of secondary metabolites in the extracts by thin layer chromatography (TLC).

Also determining the most suitable mobile phase for the plant extracts and detection method.

MATERIALS AND METHODS: Seeds of *Cyamopsis tetragonoloba L*. have been collected in the summer season from the different places of Nagpur district (State- Maharashtra, Country - India) and dried in shade. Roughly 50 kg. seeds were collected. The dried plant seeds were blended using a blender and stored in a clean glassware container until needed for analysis. The seed powder of *Cyamopsis tetragonoloba* L. stored at low moisture contents (<10%) and low temperatures [8].

Preparation of extract: Dried coarse powder of the seeds (50 gm.) was placed into the extractor of a Soxhlet apparatus and subjected to extraction by hot percolation method. The extraction was carried out by solvents of increasing polarity from n-Hexane, Ethyl acetate, Acetone, Ethanol and Methanol. The extraction was carried out with 250 ml of each solvent for a period of 48 hours. The solvent was removed under vacuum at temperature below 50°C, and then the extracts were freeze-dried.

Protocol for qualitative analysis: Phytochemicals investigation were carried out on the different extracts of the powdered specimen using standard procedure to identify the constituents as described by Mojab *et al* [9], Harborne [10], Sofowora [11], Trease and Evans [12], Siddiqui and Ali [13]. Numbers of chemical analysis test were performed to detect the presence of the active chemical constituents like tannin, saponin, quinone, phenol, steroid, flavanoid, caradiac glycosides, terpenoid [14, 15]. The test method details are as follows and their results are reported in **table 1**.

1. **Test for Tannin:** 1 ml of 5% Ferric chloride is added to the solvent free extract. The presence of Tannin is indicated by the formation of bluish black or greenish black precipitate [12].

2. **Test for Saponin:** 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for nearly 15 minutes. Formation of foam indicated the presence of saponin [13].

3. **Test for Quinone:** 1 ml of extract and 1 ml of concentrated sulphuric acid (H_2SO_4) added. Formation of red colour reported the presence of quinone.

4. **Test for Phenol:** 1ml of extract 2ml of distilled water was added followed by few drops of 10% $FeCl_3$ appearance of blue or green colour indicates presence of phenol.

5. **Test for Steroid:** 1 ml of extract dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid (H_2SO_4) added by sides of test tube. The upper layer turns red and sulphuric acid layer shown yellow with green fluoresce indicated the presence of steroids [13].

6. **Test for Flavonoids:** 1 ml of extract and a few drops of dilute NaOH were added. The intense yellow colour produced in the plant extract which becomes colourless on addition of few drops of dilute mineral acid indicated the presence of flavanoids.

7. **Test for Caradiac Glycoside:** 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of $FeCl_3$ solution. This was than underlayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicated a de-oxy sugar characteristic of cardenolides.

8. **Test for Terpenoid:** 5 ml of each extract was mixed with 2 ml of chloroform. To this, 3 ml of concentrated H_2SO_4 was added to form a layer. A reddish brown precipitate colouration at the interface formed, indicated the presence of terpenoids.

The presences of various phytochemicals in prepared extracts are reported in **table 1**.

TLC Analysis of Seed Extracts of Cyamopsis Tetragonoloba L.: Each of the aforesaid five extracts was to begin with, checked by thin layer chromatography (TLC) on analytical plates over silica gel-G. For each extracts three different solvent mixtures were used as developing systems listed in **table 2**.

In each case the TLC plates were exposed to Iodine vapour and reported spots were visualized and noted down. The pictures of each of them were depicted in **figure 1 and table 3** shows the calculated R_f values for all studied TLC systems.

TABLE 1: REPORTED PHYTOCHEMICALS IN VARIOUS EXTRACTS OF SEED POWDER OF *CYAMOPSIS TETRAGONOLOBA L.*

Phytochemicals	Extracts				
	Methanol Extract	Ethanol Extract	Acetone Extract	Ethyl acetate Extract	n-Hexane
Saponin	+	-	-	-	-
Quinone	+	+	+	+	+
Phenol	+	+	+	+	+
Steroids	+	-	+	+	+
Flavanoid	+	+	+	+	+
Caradiac glycoside	+	-	+	+	-
Terpenoid	+	+	+	+	+

Where (+) = Present, (-) = Absent

TABLE 2. DIFFERENT DEVELOPING SYSTEMS USED FOR TLC ANALYSIS OF *CYAMOPSIS TETRAGONOLOBA L.*

Developing system Number	Developing system	Ratio
System- I	Chloroform:n-Hexane	7.5:2.5
System- II	Ethyl acetate:Methanol	1:1
System- III	Chloroform:Glacialacetic acid:Methanol:Water	60:32:12:8

RESULTS: Quantitative analysis performed on different solvent extracts of Cyamopsis tetragonoloba L. seeds show the presence of Saponin, Quinone, Phenol, Steroids, Flavanoids and Terpenoids. Quinone, Phenol, Flavanoids and Terpenoids are largely reported in nearly all extracts. Saponin is reported only in Methanol extract. The results of phytochemicals analysis are reported in tabular form in table 1. The presence of reported phytochemicals compounds is then analysed by thin layer chromatography. The R_f values in three different mobile phases for various extracts are reported in Table 3 whereas figure 1 shows photographs of the studied TLC slides.

TABLE 3 : SHOWING R_F VALUES OF SEED EXTRACTS OF *CYAMOPSIS TETRAGONOLOBA* L. IN VARIOUS SOLVENT SYSTEMS

Plant Extracts	System- I	System- II	System- III
Methanol	0.980 0.406	0.945 0.351	0.948 0.794
Ethanol	0.833 0.285	0.942 0.884 0.326	0.971
Acetone	0.538	0.976 0.25	0.425
Ethyl acetate	0.615	0.857	0.564
n-Hexane	0.731	0.790 0.348	0.972 0.270

DISCUSSION: The phytochemicals screening and qualitative tests performed on the seed extracts of Cyamopsis tetragonoloba L. indicates the presence of most of phytochemicals. Qualitative phytochemicals screening is an essential step towards discovery of new drugs as it provides the information regarding the presence of a particular primary or secondary metabolite in the plant extract(s) of clinical significance.

The presence of any significant bioactive natural product indicates the necessity of separation of the compound from the mixture of compounds through suitable chromatographic techniques.

In the present study, in n-hexane extract quinone, steroids, flavanoids, caradiac glycoside, terpenoid have been found to be present. The presences of quinone, phenol, steroids, flavanoids, caradiac glycosides and terpenoid have been found in case of ethyl acetate and acetone extract.

In case of ethanol extract tannin, quinone, phenol, flavanoids, and terpenoid are confirmed. Whereas, all major tested phytochemicals are reported in methanol extract.

FIGURE 1: PHOTOGRAPHS:-TLC PROFILE OF *CYAMOPSIS TETRAGONOLOBA* L. SEED EXTRACTS

These preliminary phytochemicals investigations focus on the importance of separation of the natural compounds from their mixture as they may be used for various clinical practices. Thin layer chromatographic techniques were performed on different solvent systems. Compounds have been separated well in solvent systems of chloroform: n-Hexane, ethyl acetate: methanol and chloroform: glacial acetic acid: methanol: H_2O. Various chemical constituents give different R_f values in different

solvent systems. An idea about the polarity of various chemical constituents is also obtained while performing TLC analysis. Suitable solvent system has been chosen for performing further separation of compounds.

CONCLUSION: *Cyamopsis tetragonoloba* L. plant seeds screened for phytochemical constituents seemed to have potential as source of useful drugs and also to improve the health status of its users as a result of the presence of various phytochemical compounds that are vital for good health. Qualitative tests performed on the seed extracts of *Cyamopsis tetragonoloba* L. indicate the presence of quinone, steroids, flavanoids, terpenoid in n-hexane extract.

The presence of quinone, phenol, steroids, flavanoids, caradiac glycosides and terpenoid have been found in the extract of acetone and ethyl acetate. The ethanol extract is having quinone, steroids, flavanoids and terpenoids. In case of methanol extract tannin, saponin, quinone, phenol, steroids, flavanoids, caradiac glycosides and terpenoids are present. TLC profiling also suggests the presence of diverse kinds of phytochemicals in all those seed extracts.

REFERENCES:

1. Kays SE, Morris JB and Kim Y. "Total and soluble dietary fibre variation in (*Cyamopsis tetragonoloba* (L.) Taub.) (Guar) genotypes". Journal of Food Quality. 2006, 29(4): 383-391.
2. Kaushal GP, Bhatia IS, "A study of polyphenols in the seeds and leaves of guar (*Cyamopsis tetragonoloba* L. Taub)". J. Sci. Food Agric., 2006, 33: 461-470.
3. Khalil MM. "Biochemical and technological studies on the production of isolated guar protein". Nahrung, 2001, 45(1): 21-24.
4. Anderson E. "Endosperm mucilages of legumes". Ind Eng Chem, 1949, 41:2887-2890.
5. Mukhtar HM, Ansari SH, Bhat ZA, Naved T. "Antihyperglycemic Activity of *Cyamopsis tetragonoloba* Beans on Blood Glucose Levels in Alloxan-Induced Diabetic Rats". Pharm. Biol., 2006, 44(1): 10-13.
6. Mohamed et. al., "Biochemical studies on Plantago major L. and *Cyamopsis tetragonoloba* L". Int. J. Biodivers. and Conserv., 2011, 3(3), 83-91.
7. Khatta VK, Kumar N, Gupta PC. "Chemical composition and amino acid profile of four varieties of guar (Cyamopsis tetragonoloba) seed". Indian J. Anim. Nutr., 1988,5: 326.
8. Franklin TB, USDA Forest Service's Southern Research Station, Mississippi State, Mississippi, Woody Plant Seed Manual , 1974, 848.
9. Somolenski SJ., Silinis H and Farnsworth NR., Lloydia, 1974**,** 37**:**506-536.
10. Harbone JB, Phytochemical methods, London Chapman and Hall, Ltd. 1973**:** 49-188.
11. Sofowora A., "Medicinal plants and Traditional medicine in Africa": Spectrum Books Ltd, Ibadan, Nigeria, 1993**,** 2**,** 289.
12. Salehi SMH., Aynehchi Y, Amin GH and Mahmoodi Z., Daru, 1992, 2**,** 281-291.
13. Siddiqui AA and Ali M. "Practical Pharmaceutical Chemistry". Ist Edition. CBS Publishers and distributors, New Delhi, 1997, 126-131.
14. Ganatra SH., Ramteke AM., "TLC Analysis of Pithcellobium Dulce Seed", RJPP, 2012,4(4): 220-222.
15. Ganatra SH., Durge SP., Patil SU.; " Priliminary Phytochemical investigation and TLC analysis of *Ficus racemosa* leaves" , JCPR, 2012, 4(5): 2380-2384.

How to cite this article:
GanatraSH, Ramteke AM, Durge SP and Patil SU: Phytochemicals investigation and TLC profiling of *Cyamopsis tetragonoloba* L. seeds (Fabaceae) - Pea Family. *Int J Pharm Sci Res* 2013; 4(4); 1551-1555.