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Physicochemical and Preliminary Phytochemical Screening of Root of *Cryptolepis buchanani*

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Abstract: *Cryptolepis buchanani* Roem. and Schult. (Asclepiadaceae), a climbing tree is used as folk medicine in Southeast Asia. In Thailand, the stem of this plant is traditionally used for the treatment of inflammation, including arthritis, muscle and joint pain. The potential phytochemical components of this plant's 4.5 % ethanol extract in roots of *Cryptolepis buchanani* were isolated, in the present study. The chemical composition of *Cryptolepis buchanani* extract was analyzed using GC-MS where 10 active phytochemicals were identified, such as tannins, alkaloids, saponins, glycosides, terpenes, flavonoids, phenol, protein, and steroids.

Keywords: *Cryptolepis buchanani*, Phytochemical Constituents, GC-MS, Tannins, Alkaloids, Saponins, Glycosides, Terpenes, Flavonoids

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Introduction

Cryptolepis buchanani known as “Thao En On” has been used for treating inflammatory conditions such as muscle and joint pain (Laupattarakasem *et al.*, 2006; Mace, 1963; Bunyaprapatsorn and Chokchaicharoenporn, 1993; Wuthithammawet, 1997). Its stem extract is being used in the treatment of muscle tension, stiffness of tendons, and arthritis. (Mace, 1963; Wuthithammawet, 1997). Its leaves are used as poultices on the

inflamed area for the treatment of myalgia and arthritis (Wuthithammawet, 1997). Few studies have examined the anti-inflammatory effect of this plant and found that its extract could reduce inflammation in both *in vitro* and *in vivo* studies (Laupattarakasem *et al.*, 2006). However, scientific reports of the analgesic and chondroprotective activities of *C. buchanani* are limited.

The plant is used as conventional medicine as an anti-ulcerative, anti-inflammatory, anti-diarrhoeal, antibacterial, anti-cough, blood purifier, and lactation in women (Bhakuni *et al.*, 1969), bone fracture (Kaul *et al.*, 2003) and also curing rickets in children (Kaul *et al.*, 2003). *C. buehanani* has been reported for the presence of phytocomponents such as cryptosin (Venkateswara *et al.*, 1989), sarverogenin, isosarverogenin glycosides (Purushothaman *et al.*, 1988), new nicotinoyl glucoside (Dutta *et al.*, 1980), cryptolepain (Pande *et al.*, 2006), buehanani (Khare and Shah, 1983), and possess antioxidant, hepatoprotective (Padmalochana *et al.*, 2013), analgesic, anti-inflammatory, chondro-protective (Hanprasertpong *et al.*, 2014), immunomodulatory (Kaul *et al.*, 2014), and cardiogenic activities (Venkateswara *et al.*, 1989). The potential phytochemical components of this plant's 4.5 % ethanol extract in roots of *Cryptolepis buehanani* were isolated and the chemical composition of *Cryptolepis buehanani* extract was analyzed using GC-MS to identify active phytocompounds, such as tannins, alkaloids, saponins, glycosides, terpenes, flavonoids, phenol, protein, and steroids.

Materials and Methods

Collection of Plant materials:

Cryptolepis buehanani plants were collected from the Kanyakumari district and authenticated by Dr.V.Chelladurai (Research Officer), Botany (C.C.R.A.S), Government of India. Materials were cleaned with water and dried in the shade until a constant weight was obtained.

Procedure for Extraction:

The coarsely powdered dried root of *Cryptolepis buehanani* (50 g) was macerated for 48 h with 200 ml of 50% ethanol, with occasional stirring. After 48 h, ethanolic extract was filtered through Whatmann filter paper. The plant material was then macerated again with fresh 50% ethanol and the combined filtrate was obtained from the first and the second maceration, this was then distilled under vacuum, the temperature of distillation is

maintained between 55- 60°C. After 3-5 h the three cycles were completed, the extract was then evaporated to dryness and the total yield was noted. Further all the extracts were air dried till solid to semi-solid mass was obtained and stored in air tight container in refrigerator below 10°C. Percentage yield of ethanolic root extract of *Cryptolepis buehanani* (EECB) was 4.5%.

Preliminary Qualitative Test of EECB:

The presence of various phytoconstituents was determined by the standard qualitative methods (Brain and Turner, 1975).

Qualitative analysis of Phytochemicals:

Test for alkaloid:

(a) *Mayer's test*: To a few ml of sample, added 2 ml Mayer's reagent along the sides of the test tube. It showed the white creamy precipitate which confirmed the presence of alkaloids. (Evans *et al.*, 1997)

(b) *Wagner's Test*: A few drops of Wagner's reagent was added to the samples along the sides of the tube test. The reddish/brown color was obtained which confirmed the presence of alkaloids (Wagner, 1993).

(c) *Hager's Test*: The extract was treated with Hager's reagent (saturated Picric acid solution). A yellow precipitate was obtained confirming the presence of alkaloids.

Test for Flavonoids:

(a) *Shinoda test*: A few fragments of magnesium ribbon and concentrated HCl were added to the samples along the sides of the test tube. The appearance of red to pink color after a few minutes confirmed the presence of flavonoids.

(b) *Alkaline reagent Test*: An aqueous solution of the extract is treated with 2% sodium hydroxide solution and concentrated HCl was added. Yellow color fluorescence was obtained which confirmed the presence of flavonoids.

(c) *Beta-Smith and Metcalf Test*: To a few ml of plant extract, 1 ml of concentrated HCl was added. It was then warmed in the water bath for 15 min

and it is observed for an hour. A strong red or violet color was obtained confirming the presence of flavonoids.

Test for glycosides:

(a) Aqueous Sodium hydroxide test: The sample was treated with 1 ml of water and 1 ml of sodium hydroxide. Yellow color was obtained, confirming the presence of glycosides.

(b) Legal's Test: 50 mg of the dried extract was dissolved in Pyridine and Sodium nitroprusside solution was added and made alkaline by adding 10% NaOH. The pink color was formed confirming the presence of glycoside.

Test for phenolic compounds:

(a) Ferric Chloride Test: The dried extract (50 mg) was dissolved in 5 ml of water. To this 2 ml of 5% neutral ferric chloride solution was added. A dark green color was formed, confirming the presence of phenol (Mace, 1963).

(b) Lead Acetate Test: The dried extract (50 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate was formed which confirmed the presence of phenol.

Test for phytosterols:

Libermann-Burchard's Test: The sample was dissolved in 2 ml of acetic anhydride. To this 1 or 2 drops of concentrated sulphuric acid was added slowly along the sides of the test tube. An array of color change was observed which confirmed the presence of phytosterols (Finar, 1986)

Test for saponins:

Foam Test: The dried extract (50 mg) was mixed with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A layer of foam was observed, which confirmed the presence of saponins (Kokate, 1999).

Test for terpenoids:

Salkowski Test: To the plant extract, 2 ml of chloroform and 3 ml of conc. sulphuric acid was

carefully added along the sides of test tubes to form a layer. Reddish-brown coloration was observed which confirmed the presence of terpenoids (Harborne, 1998).

*GC-MS Analysis of *Cryptolepis buchanani*:*

GC-MS is a combination of two different analytical techniques, Gas Chromatography and Mass Spectrometry (Fig. 1). GC-MS is a reliable technique to isolate various constituents in a volatile matter (alcoholic root extract of *Cryptolepis buchanani*; Anjali *et al.*; 2009). GC-MS, with the use of internal standards, provides a multidimensional drug identification and qualitative procedure, a leading confirmation method for forensic drug testing.

Gas Chromatography (GC): Gas Chromatography is a technique used to separate drugs that might be present in a sample. The sample is injected into a long tubular chromatographic column. The drugs are swept through the column by a stream of helium gas. Components in a sample are separated from each other based on the retention time on the column. The time taken for any given component to travel the length of the column is referred to as its retention time (RT). The RT is characteristic for a given component.

Mass Spectrometry (MS): The detector employed in gas chromatography is the Mass Spectrometry (MS). As the component exits the GC column, it is fragmented by ionization and the fragments are sorted in fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component is unique and characteristic of the component. It is so specific that it is often referred to as the molecular fingerprint.

The selected ion monitoring (SIM) mode was employed during the GC-MS analysis. SIM plots of the ion current resulting from a very small mass range with only compounds of the selected mass were detected and plotted.

In recent years, Gas Chromatography-Mass Spectrometry (GC-MS) has been applied unambiguously to identify the structures of different phytoconstituents in plant extracts and

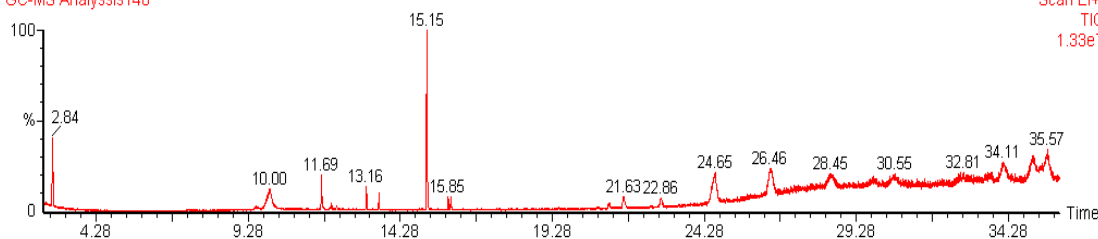


Fig. 1: GC- MS of *Cryptolepis buchanani*.

Table 1: Phytochemical profiling of *Cryptolepis buchanani*

S. No.	Test Name	Solvents	EE
1.	ALKALOIDS	Mayer's Test	+
		Wagner's Test	+
		Hager's Test	+
2.	FLAVANOIDS	Shinoda test	+
		Alkaline Reagent Test	+
		Beta smith and Metcalf test	+
3.	AMINO ACIDS	Ninhydrin Test	-
		Millon's Test	-
4.	CARBOHYDRATES	Molisch Test	-
		Benedict's Test	-
5.	GLYCOSIDES	Aqueous Sodium Hydroxide Test	+
		Legal's Test	+
6.	PHENOLIC COMPOUNDS	Ferric Chloride Test	+
		Lead Acetate Test	+
7.	FIXED OILS AND FATS	Spot Test	-
		Saponification Test	-
8.	PHYTOSTEROLS	Liebermann-Burchad's Test	-
9.	SAPONINS	Foam Test	+
10.	TERPENOIDS	Salkowski Test	+

biological samples with great success (Prasain *et al.*, 2004). GC-MS is a reliable technique to identify the constituents of volatile matter, long-chain branched hydrocarbons, alcohols acids, and esters (Anjali *et al.*, 2009).

Results and Discussion

The qualitative estimation of ethanolic extract of *Cryptolepis buchanani* in which alkaloids, tannins,

flavonoids, saponin, glycoside, terpenes, phenol, protein, and steroids were present. The carbohydrates, amino acids, fats, and phytosterol were absent as shown in Table 1.

GC-MS analysis of ethanolic extract of *Creptolepis buchanani*:

The ethanolic extract of *Creptolepis buchanani* was a complex mixture of many constituents and 10

Table 2: GC-MS analysis of *Cryptolepis buchanani*

S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Nature of compound	**Activity
1.	2.84	Propane, 1,1,3-triethoxy-	C ₉ H ₂₀ O ₃	176	9.66	Ether compound	No activity reported
2.	10.00	3-Pentanol, 2,3-dimethyl-	C ₇ H ₁₆ O	116	13.18	Alcoholic compound	Antimicrobial
3.	11.69	1,14-Tetradecanediol	C ₁₄ H ₃₀ O ₂	230	4.00	Alcoholic compound	Antimicrobial
4.	13.16	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	2.49	Plasticizer compound	Antimicrobial Antifouling
5.	15.15	Phytol	C ₂₀ H ₄₀ O	296	22.99	Diterpene	Antimicrobial Antiinflammatory Anticancer Diuretic
6.	15.95	10-Undecyn-1-ol	C ₁₁ H ₂₀ O	168	1.32	Unsaturated alcoholic compound	Antimicrobial
7.	21.63	Didodecyl phthalate	C ₃₂ H ₅₄ O ₄	502	3.47	Plasticizer compound	Antimicrobial Antifouling
8.	22.86	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	C ₁₀ H ₂₂ OS ₂	222	3.32	Sulfur compound	Antimicrobial
9.	24.62	2-Piperidinone, N-[4-bromo-n-butyl]-	C ₉ H ₁₆ BrNO	233	20.84	Alkaloid compound	Antimicrobial Antioxidant Antiinflammatory
10.	26.46	d-Mannitol, 1-decylsulfonyl-	C ₁₆ H ₃₄ O ₇ S	370	18.74	Sugar alcohol with sulfur compound	Antimicrobial Anticancer

components were identified. Phytoconstituents such as Propane, 1,1,3-triethoxy-, 3-Pentanol, 2,3-dimethyl-, 1,14-Tetradecanediol, Dibutyl phthalate, Phytol, 10-Undecyn-1-ol, Didodecyl phthalate, 2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate, 2-Piperidinone, N-[4-bromo-n-butyl]-, d-Mannitol, 1-decylsulfonyl- were identified. A total of 10 major components belonging to different chemical groups have been identified and listed in Table 2. The results revealed that the ethanolic extract of *Cryptolepis buchanani* was characterized by the presence of ether, alcoholic, plasticizer, diterpene, unsaturated alcoholic, sulphur, and alkaloid and sugar alcohol with sulphur compounds.

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