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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING OF DIFFERENT PLANT PARTS OF *PHYLLANTHUS AMARUS* SCHUM. & THONN. COLLECTED FROM CENTRAL INDIA WITH RESPECT TO THE TRADITIONAL CLAIMS FOR THEIR MEDICINAL USES

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ABSTRACT: *Phyllanthus amarus* Schum. and Thonn. is one of the highly used medicinal plants both traditionally and scientifically. Phytochemical screening is of high importance to establish the claims of medicinal uses by traditional and folk medicine practitioners. Since, environmental, climatic and soil conditions have strong effect on production and level of particular phytochemicals in a given plant part, present study was focused on the locally available plant variety and to establish the traditional claims for its medicinal uses. The qualitative and quantitative estimation of different plant parts of *P. amarus* collected from Jabalpur, India revealed the presence of high amounts of phenolic compounds, alkaloids, saponins, flavonoids and terpenoids in aerial parts of the plant i.e. stem and leaf. Roots and fruits showed high amount of phenolics and terpenoids. This is the first study that was performed with separated plant parts and not with the whole plant. The present study is an attempt to reinvestigate *Phyllanthus amarus* Schum and Thonn from Central India for the phytochemical analysis and correlate the traditional uses of the plant.

INTRODUCTION: The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted for biologically or pharmacologically screening is even smaller. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the lien of medicine and pharmacological studies.

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The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [1]. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more [2].

Due to their specialized biochemical capabilities, plants are able to synthesize and accumulate a vast array of primary and secondary chemicals useful for the plant itself as protecting against environmental stress factors. These compounds have made many plants useful also for humans, for instance, as spices and medicines etc [1, 2]. Traditionally, *P. amarus* is being used in a variety of ailments i.e. Jaundice, kidney stone, fever, fertility related problems, menstrual cycle problems. Other uses include antimalarial,

anticancer, antioxidant and various other pharmacological uses. Scientifically, antimicrobial activity is connected with alkaloids, antioxidant activity with phenolic compounds, flavonoids with variety of fever and menstrual cycle problems and terpenoids as antipyretic [1].

Phyllanthus amarus Schum and Thonn elaborates different classes of organic compounds of medicinal importance including alkaloids, flavonoids, hydrolysable tannins (Ellagitannins), major lignans, polyphenols, triterpenes, sterols and volatile oil. Many lignans were isolated from the plant viz., phyllanthin (a bitter constituent) and hypophyllanthin (a non bitter constituent) [1]. The highest amounts of phyllanthin (0.7% w/w) and hypophyllanthin (0.3 % w/w) have been reported in leaves whereas, in the stem these are in minor quantities [2].

This study investigated the phytochemicals analysis of the leaves, stem, fruits and roots of *P. amarus* collected from Central India extracted successively with solvents of decreasing polarity.

MATERIALS AND METHODS:

Identification of *Phyllanthus amarus* in fields/forests:

The young plants of *P. Amorous* creep up abundant during the rainy season. The plants were collected mainly from Jawaharlal Nehru Agricultural University campus, Jabalpur and from State Forest Research Institute campus, Jabalpur for the present study. Keeping in mind the possibility of getting more than one species of Phyllanthus in fields, all the possible plants of Phyllanthus were taken to the lab and identified for *P. amarus* based on the identification keys. Meteorological data on Jabalpur were obtained from the Meteorological Department, Jabalpur.

Collection of plant material:

The plants chosen for the study were fully grown plants not having less than 30 cm height. The plants were uprooted from the ground, taking care not to damage the roots of the plants. All the plants were washed under running tap water for one hour in order to remove adherent soil and other extraneous matter. Care was taken not to disturb the flowers and/or fruits of the plants. After washing the plants were soaked onto a newspaper to get rid of extra water. All plant parts viz. stem, roots, leaves and fruits were separated from each other manually. Fruits were picked manually and collected separately. Care was taken not to disrupt the delicate fruits.

After separation, the roots and the stem were cut into smaller pieces using a big scissor. The leaves and the fruits were dried as such. All the parts were air dried under shade for one week or longer till a constant weight was achieved. Fruits of *P. amarus* were dried in sealed envelope made from newspaper in order to preserve the fruits burst during drying.

The different plant parts of *P. amarus* were ground in a mixer grinder after drying. The powder obtained was passed through a 100 µM test sieve (Sonar, India). The remaining coarse powder was again subjected to grinding and sieving. The process was continued till no further material passed through test sieve. The fine powder obtained of less than 100 µM, was immediately stored in an airtight container for further use.

Qualitative Phytochemical screening:

For the screening, the phytochemicals were extracted sequentially with water, methanol, ethyl acetate and petroleum ether. For this, 10 g of dried plant powder was first extracted with water using cold percolation method for 48 h. After 48 h, the suspension was filtered through a multi-layered muslin cloth and the filtrate was kept refrigerated till use. The remaining powder was extracted two more times in a similar fashion to ensure complete extraction. All the aqueous filtrates were pooled and pooled extract was concentrated under vacuum. The volume was reduced to 20 ml, filtered with Whatman No. 1 filter paper and was kept refrigerated until use.

The residue after cold percolation was dried and used for further extraction sequentially with methanol, ethyl acetate and petroleum ether using a Soxhlet extractor. The extracts were concentrated to 20 ml as described above.

Qualitative tests for various secondary metabolites were performed using simple chemical methods as

described by Trease and Evans [1], Harborne [5] and Thimmaiah [1].

Quantitative phytochemical tests:

Total Phenolic content:

The total phenolic content was determined by the Folin-Ciocalteu method described by Wu et al. (2003) with some minor modification. 1.5 ml of Folin-Ciocalteau reagent (SRL, India) and 1.2 ml of 75% (w/v) sodium carbonate solution was taken in test tubes. 0.3 ml of plant methanolic extract from different parts of *P. amarus* was added to the tubes. The tubes were vortexed for 15 sec and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm with the spectrophotometer (EI, India). Results were expressed as milligram of tannic acid equivalent per gram of extract weight using standard curve of tannic acid ($R^{2=}0.98$).

Determination of alkaloids: [5]

Five grams of the plant sample were placed in a 250 ml beaker and 200 ml of 10% diethyl ether in ethanol was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH_4OH was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate, alkaloid, was dried and weighed. The percentage of alkaloid was calculated as difference in weight.

Determination of flavonoids: [1]

Ten grams of plant sample were repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage of flavonoids was calculated as weight of extracted dry matter.

Determination of saponins: [11]

Twenty grams of plant sample were weighed into a conical flask. To the flask, 100 ml of 20% ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. It was then filtered with a Whatman No. 42 paper. The residue was re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over a water bath at about 90°C. The concentrated extract was then transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded. This purification process was repeated three times, and all aqueous layers were pooled.

60 ml of n-butanol was added and the combined n-butanol extract was washed twice with 10ml of 5% NaCl. The remaining solution was then heated on a water-bath in a pre-weighed 250 ml beaker. After evaporation the residue was dried in an oven to a constant weight. The percentage of total saponins was calculated by difference in weight.

Determination of Total Terpenoids: [11]

Ten grams of plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.

RESULTS:

The plants of *P. amarus* were collected from the Jabalpur region of Central India during the rainy season of 2013 when these plant creep up in the wild. The qualitative phytochemical screening for secondary metabolites showed presence of various phytochemicals in different extracts for each plant part. **Table 1** shows the presence of alkaloids in ethyl acetate extracts of leaf and root of *P. amarus*. Saponins were detected in stem (aqueous and methanolic extracts) while flavonoids were detected in all plant parts but roots. Aqueous extracts from all plant parts showed positive tests for resins.

Presence of tannins was detected in all extracts of all plant parts except ethyl acetate extract of leaves and methanolic extract of root. The stars were found in stems and fruit only. Cardiac glucosides were completely absent in all four plant parts. Triterpenes were detected in good amounts in all plant parts. Caumerins were completely absent while anthraquinones were detected only in aqueous extract of the fruit of *P. amarus.*

TABLE 1: QUALITATIVE PHYTOCHEMICAL ESTIMATIONS IN FOUR PLANT PARTS OF *PHYLLANTHUS AMARUS* EXTRACTED SUCCESSIVELY WITH FOUR SOLVENTS. AQ= AQUEOUES, MEOH = METHANOLIC, EA = ETHYL ACETATE AND PE= PETROLEUM ETHER EXTRACT.

Qualitative test	Stem				Leaf				Root				Fruit			
	Aq	MeOH	EA	PE	Aq	MeOH	EA	PE	Aq	MeOH	EA	PE	Aq	MeOH	EA	PE
Alkaloids																
Mayer' test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dragendroff's	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Wagner's test	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
Saponins																
Foam test	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavinoids	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+
Resins	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Tannins																
Gelatin test	-	+	+	-	+	-	-	+	+	-	+	-	-	-	-	-
Lead acetate	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+
Ferric chloride	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-
Sterols																
Salkowski's test	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-
Liebermann's test	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Cardiac glucosides																
Keller-Killiani test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+
Caumerins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

When pharmacologically important phytochemicals in different plant parts of *P. amarus* were quantified, total phenolic content was found to be good amounts. The fruit showed maximum phenolic content as 194.2 mg equivalent tannic acid g^{-1} followed by leaves, roots and stem of *P. amarus.* Alkaloids were found higher in roots than in leaves. Alkaloids were not in quantifiable range in stem and fruits. Flavonoids were found highest in stem followed by leaf and fruit, but could not be detected in roots of *P. amarus.* Saponins were quantified only in the stem and not in other plant parts. Terpenoids were detected highest in stem (19.2 mg g^{-1}) followed by leaves, roots, and fruits respectively **(Table 2)**.

TABLE 2: QUANTITATIVE ESTIMATION OF PHARMACOLOGICALLY IMPORTANT SECONDARY METABOLITES IN DIFFERENT PARTS OF *PHYLLANTHUS AMARUS*.

#	Test	Quantity (in mg g^{-1})			
		Leaf	Root	Stem	Fruit
1.	Total Phenolic	171.2	174.1	165.1	194.2
2.	Alkaloids	1.2	1.9	-	-
3.	Flavonoids	31.5	-	49.5	30.2
4.	Saponins	-	-	12.5	-
5.	Total Terpenoids	16.7	14.8	19.2	13.6

DISCUSSIONS: *P. amarus* is widely distributed in all tropical and subtropical regions of the world. Paleobotanical studies have not found the exact geographic origin of this plant. It is indigenous to the rainforests of the Amazon and other tropical countries like India, China, and Bahamas [3]. *P. amarus* is often used in the traditional system of medicine for a variety of ailments, including dropsy, diabetes, jaundice, asthma and bronchial infections. In the Ayurvedic system of medicine, it is used in problems of the stomach, genitourinary system, liver, kidney and spleen [3]. The whole plant is used in gonorrhea, menorrhagia and other genital affections. It is useful in gastropathy, diarrhea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds, It is also used as a good tonic [3]. The Spanish name 'chanca piedra' means "stone breaker or shatter stone". In Southern America, 'chanca piedra' has been used to eliminate gall bladder and kidney stones and to treat gall bladder infections [16], cardiovascular problems [3], and also a remedy around the world for influenza [3].

Phenolic compounds are secondary metabolites in plants that are involved in a number of metabolic

pathways and are essential for plant growth and reproduction, and as protecting agents against pathogens. Phenolic compounds may play an important role in preventing chronic illnesses such as cardiovascular disease, certain type of cancers, neurodegenerative disease, and diabetes [3].

In plants, these metabolites and their derivatives play an important role in cell wall integrity and defense against pathogens [3]. Flavonoids and other polyphenols belong to the recently popular phytochemicals, chemicals derived from plant material with potentially beneficial effects on human health. The antioxidant activity of flavonoids is efficient in trapping superoxide anion (O_2), hydroxyl (OH), peroxyl (ROO) and alcohoxyl (RO) radicals [3]. Flavonoids has been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti- cancer activities and anti- microbial effects, hence, flavonoids may have a contributory effect to its fertility properties and other pharmacological effects the plant possesses [4, 5].

Tannins are formed by polymerization of quinone units and one of their molecular actions is to complex with proteins. Thus the mode of antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins etc. [3] Ellagitannins (geraniin and corilagin) were shown to be the most potent mediators of the antiviral HIV activity. Phyllathin and hypophyllanthin present in *P. amarus* exhibited antitumor, hepatoprotective and antioxidant effects [18]. Presence of phenolic group compounds in all plant parts of *P. amarus* from Jabalpur makes sure that this plant can be used for the traditional uses i.e. antioxidant and anticancer activities.

Saponins are being used commercially as dietary supplements and nutraceuticals. Saponins are expected to lead to hydrolysis of glycoside from terpenoid and hence reduce the toxicity associated with the intact molecule [3]. Alkaloids are generally toxic to other organisms. They often have pharmacological effects and are used as medications, as antimicrobial, antipyretic, local anesthetic and stimulant, psychedelic, analgesic, antibacterial, anticancer, antihypertension agent, the cholinomimetic, anticholinergic, vasodilator antiarrhythmia, antiasthma and antimalarial [3]. The presence of alkaloids in *P. amarus* confirms its uses as antipyretic, antimalarial and analgesic drug. Alkaloids and tannins may also contribute to the plant's effects as antimalarial, anti- diarrhoea and analgesic agents. Terpenes are a large class of naturally-occurring organic compounds and are major constituents of plant resin and essential oils extracted from plants. *P. amarus* collected from Central India were found to possess good amount of terpenoids.

CONCLUSIONS: Use of indigenous drugs of plant origin forms a major part of complementary and traditional medicine. Global demand for medicinal plant products such as pharmaceuticals, phyto-chemicals, nutraceuticals, cosmetics and other products is on the increase [3]. Plant active pharmacological ingredients tend to change with time and space. The age of the plant, climatic conditions, variety of the plant and soil chemistry are some of the renowned factors that influence the production of secondary metabolites in a given plant. It is hence very important to study the local cultivars of important medicinal plants so that their use in herbal drugs and as traditional and folk medicine can be more effective.

The chemical review on genus *Phyllanthus,* reveals the presence of sterols and/ or terpenes, lignans, flavonoids, polyphenolic compounds and tannins, in addition to minor alkaloids [18]. The present study is in line with the earlier findings. The present study thus envisages that *P. amarus* collected from Central India possess major phytochemicals that can correlate well with the traditional medicinal claims for this plant.

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