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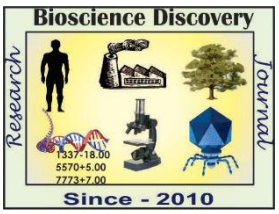

Phytochemical Studies and *in vitro* α-amylase inhibitory activity of leaves of *Guilandina bonduc* L., among Nimar Region of Madhya Pradesh

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Abstract

The present study investigates the impact of various organic solvents on the phytochemical content, antioxidant activity and *in vitro* α-amylase inhibitory activity of various crude extracted obtained from the leaves of *Guilandina bonduc* L., collected from Nimar Region of Madhya Pradesh. Results of phytochemical studies indicate that the crude methanolic extract of leaves exhibited the highest extraction yield of crude phytochemicals (29.2 %), while the petroleum ether extract showed the lowest yield (2.2 %). Phytochemical screening of the various crude extracts obtained from the leaves revealed variations in phytochemical composition. The highest phenolic content was observed in methanolic extracts (50.00 mg/g), alongside the maximum flavonoid content (0.09 mg/g) as compared to various extracts of leaves. Antioxidant activity results showed the percentage of DPPH radical scavenging activity of various extracts of leaves was dose dependent. Among them, highest antioxidant activity was obtained in crude ethyl acetate extract of leaves (69.45 %). However, *in vitro* α-amylase inhibitory activity result showed the highest percentage inhibition in crude ethyl acetate extract (74.03%) while lowest activity was reported in methanolic extract (8.791). Thus, this finding concludes that the crude methanolic extract and ethyl acetate extracts of leaves of *Guilandina bonduc* L. showed diverse phytochemical compositions among them with great antioxidant activity as well as ***in vitro*** inhibition against α-amylase.

INTRODUCTION

Guilandina bonduc L. (*Caesalpinia bonduc* (L.) Roxb.) is a wild, thorny perennial medicinal plants belonging to family Caesalpiniaceae. It is commonly known as Karanjuwa or Kanta Karanj in Madhya Pradesh. This plant is widely distributed in hotter parts, coastal areas, deltaic, eastern, western, southern parts of India and predominantly distributed in tropical and subtropical regions of Asia. Literature revealed that all parts of *Guilandina bonduc* L. have medicinal properties and considered as a promising source of drugs in traditional systems of medicines. Leaves, seeds, stem, bark, nuts and roots are useful as herbal medicines. Ethnobotanical studies carried on *Guilandina bonduc* L. revealed that it has been widely used as a traditional ethno-medicine to treat fever, hydrocele, diabetes and other disease conditions by tribal communities. The traditional practitioners residing in the vicinity of Western Ghats of Karnataka are using the leaves and stem bark to cure jaundice and liver disorders (Kumar, *et al*., 2018). The leaves alleviate *Kapha* and *Vata*, are emmenagogue febrifuge and anthelmintic and are useful in the treatment of fevers, splenomegaly, hepatomegaly, piles, intestinal worms, elephantiasis, amenorrhea, dysmenorrhea, and pharyngodynia (Prasad, *et al*., 2010). The leaves of the plants have the activities as liver and gastric tonic, fungicidal, anticonvulsant (Devi, *et al*., 2019). The potential of medicinal values shows that *Guilandina bonduc* L. contains number of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, steroids and triterpenes etc.

Hence, to confirm the ethnobotanical usages of leaves part of *Guilandina bonduc* L. by tribal communities of Madhya Pradesh, a systematic phytochemical study carried out to investigate the quality and quantity of phytochemicals present in various crude extracts of *Guilandina bonduc* L. leaves.

MATERIALS AND METHODS

2.1 Plant material- During the months of March to May, fresh and healthy leaves of *Guilandina bonduc* L., were collected from Sendhwa city (21°40'41.0"N 75°05'44.1"E) of Barwani district in the Nimar Region of Madhya Pradesh. The study was conducted at the Dept of Botany, Govt. Holkar Science College, Indore, India.

2.2. Plant extraction and extract preparation- The leaves samples were washed under tap water and air dried in shade for 10 days and then in oven at 60°C until completely dried, then grinded to fine powder by using electric blender and stored in clean labeled airtight bottles. Four different organic solvents (methanol, ethyl acetate, chloroform and petroleum ether) were used as extracting solvent for preparing crude extracts of leaves by solvent extraction method; leaves samples were extracted by soaking 10 g dry leaves powdered material in 150 ml of various organic solvents at its respective boiling temperature for 24 hs in Soxhlet apparatus, filtered through Whatman no.1 filter paper followed by cotton wool. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40°C. Extracts were reconstituted in known amount of methanol to obtain methanol extract of known concentration. The stock solution of each extracts of leaves (1 mg/ml) was prepared by dissolving a known amount of dry extract in 100 ml of methanol. The different working solutions of various crude extracts were prepared from the stock solution using suitable dilution for further investigations such as crude phytochemical yield, phytochemical screening test, estimation of total phenolic content, flavonoid content, antioxidant activity and *in vitro* α-amylase inhibition activity.

2.3 Determination of extraction yield- The extraction yield (%) of various organic solvents extracts of leaves was calculated as follows:

$$\textbf{Extraction yield}\ (\%) = \frac{\textbf{weight of the extract after evaporating solvent and freezing drying}}{\textbf{dry weight of the sample}}$$

2.4 Preliminary phytochemical analysis- The various organic solvents extracts of leaves were subjected to various phytochemical tests to determine the active constituents present in samples.

2.4.1 Hager's test- A few drops of Hager's reagent were added to a 2 ml of extract of leaves of tested samples. Bright yellow precipitate formation indicated the existence of alkaloids.

2.4.2 Folin test for phenol- To 2–3 ml of extract of leaves, 1 ml of Folin's reagent was added. Violet/brown coloration of the solution indicates the presence of phenol in the tested leaves extracts.

2.4.3 Shinoda test for flavonoids- To 2–3 ml of extract of leaves, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. Pink red or red coloration of the solution indicate the presence of flavonoids in the tested extract of leaves.

2.4.4 Cardiac glycosides (Keller-Kiliani test)- Add 2 ml of 5% glacial acetic acid, one drop of 5% ferric chloride, 5 ml of extract of leaves, and concentrated sulfuric acid. A brown ring of the interface indicated the presence of cardiac glycosides.

2.4.5 Test for phytosterols- The extract of leaves was treated first with chloroform (2 ml) and then concentrated sulfuric acid (2 ml). To this solution, dilute acetic acid (few drops) and 3 ml of acetic anhydride was added. Appearance of bluish green color showed the presence of phytosterols.

2.4.6 Salkowski test for terpenoids- The extract of leaves (5 ml) was mixed with chloroform (2 ml), and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.

2.4.7 Saponins (form test)- The extract of leaves (2 ml) was mixed with 5 ml distilled water and shaked violently. If stable foam forms, saponins will be present in the samples.

2.5 Total Phenolic Content (TPC) determination assay- The TPC was evaluated using spectrophotometrically according to the Folin–Ciocalteu method with slight modification. 0.1 ml of crude extracts (1 mg/ml) of each tested samples was combined with 0.5 ml of Folin–Ciocalteu reagent (Ainsworth and Gillespie, 2007). 2.9 ml of distilled water to each standard and sample tube was added; this was then vortexed for few seconds, covered and incubated for exactly 30 min at room temperature. Then, 2.0 ml of a 2% Na_2CO_3 solution was added, absorbance at 750 nm was measured. Gallic acid (standard phenolic compound-1 mg/ml) was utilized as a standard reading. The standard graph was compared to the sample readings. The TPC of extracts was expressed as mg/g (dry weight of leaves). 2.6 Total Flavonoid Content (TFC) determination assay.

The TFC of extracts of leaves samples was determined using the aluminum chloride colorimetric technique (1mg/ml). 0.1 ml of crude extracts of leaves, 0.3 ml of 10% aluminum chloride, 0.15 ml of 5% sodium nitrate, 1 ml of 1 M sodium hydroxide, and 2.5 ml of distilled water were combined. The absorbance of the reaction mixture was detected at 510 nm. The calibration curve was based on quercetin (standard flavonoid molecule at 1 mg/ml). The standard graph was compared to samples. The total flavonoid concentration of samples was expressed in mg/g (dry weight basis of leaves) (Chang *et al*., 2002).

2.7 Antioxidant activity in vitro (DPPH radical scavenging activity)- The scavenging ability of natural antioxidants of crude extracts of leaves towards the stable free radical DPPH was measured by method of Shimada *et al*., (1992). Add 5 ml of DPPH (0.1 mM) solution in 0.1 ml of crude leaves extracts in different concentration and make up to 6 ml by methanol (0.5ml). The mixture was shacked vigorously, allowed to stand at room temperature for 30 minutes in dark. Changes in absorbance of the samples were measured at 517 nm in UV ectrophotometer. Ascorbic acid (0.1mg/ml) was used as the standard.

Radical scavenging activity (%) = $A_C - A_S / A_C \times 100$

Where A_C = absorbance of control

A_S = absorbance of sample

2.8 *In vitro* α-amylase inhibitory activity by DNSA method- α-amylase inhibitory activity of the extracts of tested samples was estimated *in vitro* by the procedure as described by Banerjee *et al*., (2017) and Aleixandre *et al*., (2022) with slight modifications. The working solutions of the selected samples were prepared with the concentration of 10 ppm. Each 1 ml of sample was treated with 0.5 ml of 0.5% α-amylase solution and 1 ml of sodium phosphate buffer in test tubes and incubated for 10 min. After 10 min, 1 ml of 1% starch solution was added to each test tube and again incubation was provided for 20 min. The reaction was then terminated by adding 1 ml of DNSA solution and the tubes were kept in boiling water bath for 15 min. After 15 min, the tubes were allowed to cool at room temperature and the absorbance was measured at 545 nm. Blank was prepared by following the same procedure with no sample and 1% starch solution. Positive control showing enzyme activity was prepared by using 1 ml of 0.01% EDTA solution instead of sample. Percentage inhibition of α-amylase was calculated by the formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.9 Data analysis- Quantitative and graphical data were analyzed using Microsoft Excel Package. The results of each series of experiments (performed in triplicates) were expressed as the mean ± standard deviation. Significant differences are obtained when *P*-value was <0.05.

3. RESULTS AND DISCUSSION

3.1 Extraction yield- Quantitative estimation of the percentage yield of various extracts of leaves obtained from *Guilandina bonduc* L. studied is summarized in Table 1. The results showed that the maximum extraction yield of phytochemicals was reported in methanolic extracts of leaves (29.2 %) and it was followed by ethyl acetate extract (7.8%). However, minimum yield was noted in petroleum ether extract (2.2%). Phytochemical extraction yield of *Guilandina bonduc* L. leaves showed maximum yield in methanol can be explained by the fact that the extractable substances have more affinity for the methanol solvent as compared to other solvents. Other findings revealed that the extraction yield of *Guilandina bonduc* L. leaves was 13.58% reported in hydroethanolic extract (Ouattara *et al*., 2020). Pandey and Lokesh (2019) also evaluate *in vitro* antioxidant activities, phytochemical analysis and HPLC analysis of ethanol extract of leaves of *Guilandina bonduc* L. collected from Bhopal region of Madhya Pradesh stated that the yield of extracts obtained from different samples using Pet. ether, chloroform, ethyl acetate, ethanol, aqueous as solvents showed 10.6 % yield of extracts in ethanol solvent which was followed by water extract (7.14) and lowest % yield was obtained in Pet. Ether (2.14 %).

3.2 Phytochemical screening (Qualitative analysis)- The present study revealed that the various extracts of *Guilandina bonduc* L. leaves showed variation in terms of its phytochemical constituents which are tabulated in Table 2. The results indicated that the alkaloids, phytosterols, saponins, cardiac glycosidase, phenol, and flavonoid were the phytoconstituents found in all the tested samples of methanolic extracts which was followed by ethyl acetate. However, in case of petroleum ether extract only alkaloids, phenol and flavonoids were showed its presence as compared to rest of samples. Similar findings were also done by other researcher stated that qualitative phytochemical analysis of the crude powder leave of *Guilandina bonduc* L. showed variation in terms of its phytochemicals constituents in various extracted solvents (Pandey and Lokesh 2019). Kundu *et al*., (2011) also did preliminary phytochemical analysis of *Guilandina bonduc* L. leaves where result of preliminary phytochemical analysis showed alkaloids,

Table 1 % Yield of crude phytochemicals of leaves extracts of *Guilandina bonduc* L.-

Extracts of *Guilandina bonduc* L. leaves	Weight of leaves used (g)	Yield (g)	Yield (%)
Methanol	10.00	2.92	29.2
Ethyl acetate	10.00	0.78	7.8
Chloroform extracts	10.00	0.40	4.0
Petroleum extracts	10.00	0.22	2.2

tannins and proteins were found to be present in aqueous extract where as steroids were also found in alcoholic extract. Preliminary phytochemical screening of these plant materials revealed the presence of alkaloids, saponins, flavonoids, steroids, phytosterols and carbohydrates in methanol and ethanol extracts (Raghav and Singh 2014).

Table 2 Preliminary qualitative phytochemical analysis of various extracts of *G. bonduc* leaves.

Plant constituents	Methanolic extract of leaves	Ethyl acetate extract of leaves	Chloroform extract of leaves	Petroleum ether extract of leaves
Alkaloids	+++	+++	++	+
Phytosterols	+	+	+	-
Cardiac glycosidase	++	++	−	−
Terpenes	++	+	−	-
Saponins	+	+	+	-
Phenol	+++	++	+	+
Flavonoid	+++	++	+	+

Notes. +: present (mild amount), ++: present (moderate amount), +++: present (large amount), −: absent, based on the power of generated color reaction.

3.3 Determination of total phenolic content & total flavonoid content-

The Folin–Ciocalteu technique is used in this study to determine the TPC of the various extracts of leaves of *G. bonduc* L. TPC was estimated from gallic acid standard curve (y =0.0006x + 0.0034) and the results were represented in mg of gallic acid equivalent (GAE). Table 3 shows that the highest amount of TPC was obtained in methanolic extract (50.3 ± 1.11 mg GAE/g) which was followed by chloroform extract (7.2 mg ± 0.9 GAE/g). However, lowest TPC was reported in petroleum ether extract (2.0 mg ± 1.01 GAE/g). Similarly, TFC was estimated from the quercetin standard curve (y=1.41 x - 0.096) and the results were expressed as mg of quercetin equivalent (Table 3). Total flavonoid concentration in various extracts of leaves of *G. bonduc* L. revealed that the methanolic extract had the highest flavonoid content (0.09 ± 0.67 mg QE/g), followed by the ethyl acetate extract (0.08 ± 0.03 mg/gm). However, the lowest flavonoid content was observed in both the chloroform (0.02 ± 0.01) and petroleum ether extracts respectively (0.02 ± 0.2). Similar results were found by Ouattara *et al.*, (2020) reported that the phenolic compounds contents in different hydroethanolic extracts *G. bonduc* L. leaves was 59.11 ± 1.89 mg EAG while flavonoids compounds contents was 13.45 ± 2.82 mgEQ. In another study, Pandey and Lokesh (2019) also evaluated total phenolic, flavonoids and alkaloids content of ethanolic leave extract of *G. bonduc* L. which was to be 0.647, 0.941and 0.369mg/100mg respectively.

Table 3 Total phenolic and flavonoid contents of various extracts of *G. bonduc* leaves.

Extracts of G. bonduc L. *leaves*	Total phenolic (mg GAE/g)	Total flavonoid (mg QRE/g)
Methanol	50.3 ± 1.11	0.09 ± 0.67
Ethyl acetate	5.9 mg ± 0.7	0.08 ± 0.03
Chloroform	7.2 ± 0.9	0.02 ± 0.01
Petroleum ether	2.0 ± 1.01	0.02 ± 0.02

Values were expressed as mean ± SD (n = 3).

3.4 Antioxidant activity in vitro (DPPH radical scavenging activity)

The antioxidant activity of leaf extracts has been studied by its ability to reduce DPPH. Interaction of antioxidant compounds with DPPH is based on the transfer of hydrogen atom or electron to DPPH radical and converts it to 1, 1- diphenyl-2- picrylhydrazine (Sreelata and Padma 2009, Rahman *et al*., 2015). The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which demonstrates the scavenging activity (Akar *et al*., 2017). The results of this study showed that various extracts of *C. bonduc* leaves exhibited free radical scavenging activity in a dose-dependent manner from 62.5 to 1000 µg/ml. The ethyl acetate extracts of *G. bonduc* L. leaves had a higher DPPH free radical scavenging activity compared to the chloroform extracts and methanol extract. However, lowest DPPH free radical scavenging activity was observed in petroleum ether (Table 4).

Present finding results were compared with other published result stated that *G. bonduc* L. extracts showed effective DPPH radical scavenging in various extracts of leaves. A dose dependent activity with respect to concentration was observed and free radical scavenging activity hold considerable antioxidant potential. (Pandey and Lokesh 2019). Antioxidant activities of different hydroethanolic extracts of *G. bonduc* L. leaves was IC_{50} = 52 µg/ml (Ouattara *et al*., 2020). In another study the methanol extract of *G. bonduc* L. leaves showed significant antitumor and antioxidant activities in Ehrlich ascites carcinoma - bearing mice (Gupta *et al*., 2004). Also, the work done by Sekar et al., (2011) on *G. bonduc* L. leaves gave an IC_{50} greater than 40 µg/ml with the methanolic extract. Several reasons could explain the differences observed between these results, in particular the origin of the plant, the organ used, the methodology and the reagents used.

Table 4 DPPH scavenging activity of various extracts of *G. bonduc* L. leaves

Conc. µg/ml	Various extracts of *G. bonduc* L. leaves			
	Methanolic extract	Ethyl acetate extract	Chloroform extract	Petroleum ether extract
	RSA%	RSA%	RSA%	RSA%
62.5	36.25255	38.08554	31.77189	31.36456
125	42.97352	42.97352	33.19756	33.40122
250	44.80652	49.6945	43.38086	33.80855
500	54.78615	59.2668	54.17515	44.60285
1000	59.8778	69.4501	63.95112	51.12016

3.5 In vitro inhibitory α-amylase assay- The results in Table 5 showed *in vitro* α-amylase inhibitory activity of various extracts of *G. bonduc* L. leaves showed that the highest percentage of α-amylase inhibition activity was found in methanolic extract of leaves (87.91 %) which was followed by ethyl acetate extract of leaves (74.03%). However, the lowest percentage α-amylase inhibition activity was found in chloroform extract of leaves (54.06). When these findings were compared with those of other studies, it was discovered that a study by Shanmugapriya *et al*., (2024) investigated anti-diabetic and free radical scavenging activity of phytochemicals from *G. bonduc* L. seeds inhibit the enzyme responsible for glucose metabolism and so maximum inhibition was observed in 0.25 mg/mL. *G. bonduc* L. subjected to *in vitro* analysis of antidiabetic effect by alpha-amylase and alpha-glucosidase inhibitory assay showed highest alpha-amylase and alpha-glucosidase inhibitory activity as compared to other samples (Sachan *et al*., 2019). The highest inhibition i.e. 87.26% was observed at a concentration of 9mg/mL with the aqueous extract of seeds of *C. bonducella* (Bhutkar and Bhise 2012).

Table 5 *In vitro* % inhibitory activity of α-amylase of various extract of *G. bonduc* L. leaves.

Extracts of *C. bonduc* leaves	α-amylase inhibition activity (%)
Methanol	87.91
Ethyl acetate	74.03
Chloroform	54.06
Petroleum ether	54.97

4 CONCLUSION

The study highlights the diverse phytochemical compositions and bioactive characteristics in various extracts obtained from *G. bonduc* L. leaves. Significant differences have been found in the phytochemical composition, DPPH radical scavenging activities, TPC, TFC, and *in vitro* α-amylase inhibitory activity among different extracts. Further studies are needed with taking consideration for methanolic extract and ethyl acetate extracts of *G. bonduc* L. leaves to isolate characterize and elucidate the structure of the bioactive compounds of this plant for industrial drug formulation.

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