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PHYTOCHEMICAL AND PHYSIOCHEMICAL SCREENING OF GYMNANTHEMUM AMYGDALINUM (DELILE) SCH.BIP. EX WALP

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Abstract: Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp. (Asteraceae), better known by its former name *Vernonia amygdalina* Delile, is a small shrub with wide medicinal properties. The aim of this work is to screen the extracts of *Gymnanthemum amygdalinum* leaves for phytochemical and physiochemical composition mainly to establish its earlier claimed usage as traditional and modern medicine. The preliminary phytochemical analysis of ethanol and petroleum ether extracts showed the presence of tannins, flavonoids, alkaloids, terpenoids, and carbohydrates. The phytochemical compounds especially alkaloids, flavonoids, tannins, saponins, glycosides and carbohydrates were detected in both pet ether and ethanol extracts of leaves. Quantitative determination of the phytochemical compounds found in the barks and leaves of the tree revealed that the extracts had significantly higher values of alkaloids, flavonoids and glycosides than barks. Saponins and anthraquinnes were found to be significantly more in barks than in leaves. The total ash, water soluble, acid insoluble and sulphated ash in leaves were measured to be 12.14%, 5.53%, 2.04% and 14.02% respectively. The results obtained were remarkable and the plant can be further explored in the treatment of oxidative stress and other related disorders.

Index Terms - *Gymnanthemum amygdalinum*, phytochemical composition, physiochemical composition, quantitative analysis, Alkaloids, Terpenoids and Flavonoids.

I. INTRODUCTION

Medicinal plants form an important component of flora and are widely distributed in the world. The pharmacological evaluation of substances from plants is an established method for the identification of lead compound swhich can result to the development of novel and safe medicinal agents. Medicinal plants are composed of some certain organic compounds called phytochemicals which produce definite physiological actions in the human body and these bioactive substances include but are not limited to tannins, alkaloids, terpenoids, steroids and flavonoids (Edoega *et al*., 2005). The development of pharmaceutical products necessitates an exhaustive investigation of medicinal plants to improve our knowledge about their biological activities and the phytoconstituents responsible for them (Jothy *etal*., 2012). Furthermore, the need for comprehensive investigations in this area is more evident owing to the fact that only a limited number of medicinal plant species have received complete scientific inspection (Mazumder*etal*.,2008). Phytochemicals are naturally occurring chemical ,biological and active compounds found in plants that are of benefit to human health apart from those that act as macronutrients and micronutrients (Jasiem,2016). Various bioactive compounds such as flavonoids, phenols, alkaloids, glycosides, saponins, and anthraquinones shield plants from disease, environmental damage such as contamination, stress ,drought, pathogenic attack and contribute to fragrance, color and flavor (Kotche etal., 2016; Akintola *et al*., 2020). Phytoconstituents from MAPs have excellent antioxidant and anti- glycation activities prevent this auto-oxidation and helps in preventing complications in diabetes (Mata *et al*., 2023). Over the last few decades, recognition of herbal treatments in the treatment of various diseases and disorders has increased globally due to therapeutic efficacy, safety, minimal adverse effects and low cost. Therefore, it is highly important for the cutting-edge research to

evaluate the pharmacological potential and identify the active compounds, as these scientific findings are needed for future remedy development industry, for proving the efficacy and standardizing the herbal medicines. Medicinal plants ,aromatic plants and their products are being employed in the management of various metabolic disorders since time immemorial .Research on phytoconstituents from various botanicals showed that they regulate the oxidative stress damage by scavenging the free radicals and reactive oxygen species (Ames *etal*.,1993;Pérez*etal*.,2021; Devi*etal*.,2023).Previous studies suggest that there is a correlation between disease incidence and diet where, the risk of occurrence of degenerative diseases is less with higher intake of foods rich sources of antioxidants (Ghadermazietal.,2017).Many herbal products are used as in fusions as home based remedies to be protected from various diseases and all these plants and products have excellent anti-oxidative properties (Wang
etal., 2023; Wu*etal*.,2023; Chaughule and Barve, 2024).

Gymnanthemum amygdalinum (*G. amygdalinum*) belongs to the family Asteraceae and is commonly known as Bitter leaf. It is an evergreen shrub or small tree that is much-branched and grows up to 10 m tall 40 cm in diameter and found mostly in tropical regions. Young leafy shoots are edible, eaten as a potherb or added to soups. Leaf decoctions are used in the treatment of fever, malaria, scabies, diarrhea, cough, dysentery, headache, stomach pains, and hepatitis. It is also a laxative and fertility inducer. It possesses various pharmacological activities. It is for constipation, diarrhea, skin wounds, scabies, ascarias is, tonsillitis, fever, andmalaria(Swamy*etal*.,2015;Alara*etal*.,2017;Kaur*etal*.,2019).Thepresentstudyaimsatevaluatingtheanti-oxidantand anti-diabetic potential of extracts from leaves of *G. amygdalinum* and we here in report the results of our investigative study.

II. MATERIALS AND METHODS

Solvent extraction

Theleavesof*G.amygdalinum*weresubjectedseparatelytosolventextractionusingpetroleumetherandethanolandthe extracts were stored under nitrogen atmosphere at 4°C for further use.

Phytochemicals screening

The phytochemicals screening was done in accordance with the standard qualitative chemical methods (Trease and Evans, 2009). Both the solvent extracts from leaves were screened for the existence of carbohydrates, alkaloids, Terpenoids, Anthraquinones, Tannins, monoterpene alcohols, and flavonoids

Test for Tannins

0.5 g of crude plant extract diluted in Water (20ml) and boiled then filtered .$Fecl_3$(0.1%)was added to the filtrate and the blue to black colour indicated tannins in the samples.

Test for Anthraquinones

This was performed as per the method of Borntrager 's. Extract of 0.5 g was taken and mixed with benzene(2ml) and allowed for filtration after shaking. Then to the filtrate Ammonium solution (10ml; 1%) was added. The Mixture was shaken for 1 min and the colour change was observed. Anthraquinines can be confirmed by the appearance of violet colour at the lower of phase.

Test for Glycosides

Extract of 1gm was taken and dissolved in $FeCl_3$ (0.5ml) and 4ml acetic acid followed by Conc. Sulphuric acid (2ml). Brown ring signify that the sample consists of glycosides.

Test for Saponins

1 g of extract was taken in the test tube and added sufficient water by shaking the tube, then heated. Frothing observed in the tube indicates the presence of the saponins.

Test for Flavonoids

To the 0.5g of extract, 1ml of HCl and Mg chip was added and observed for colour change. The colours red /red crimson/ orange/crimson Magenta colour confirms flavonoids in the sample.

Test for Steroids

To the extracts (0.5 g), added 2mleach of chloroform and Con. sulphuric acid. The grass green colour indicates steroids in the extract.

Test of Phenols
To the plant extracts (0.5 g) added 1mlof$FeCl_3$. The bluish black colour indicates phenols in the sample.

Test for Alkaloids
This was performed as per Hager's test .HCl (4ml) was addedto1gmofextractandfiltered.Subsequentlysaturatedpicricacid was added in a drop wise manner. Yellow colour precipitate indicates alkaloids in the extracts.

Test of Terpenoids
The extract (5ml) was mixed with chloroform (2ml), and concentrated sulphuric acid (3ml) was carefully added to forma layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Estimation of total Phenolic content
The total phenols present in all the extracts were determined by method described in the literature (Ainsworth and Gillespie, 2007) and inquintuplicate.To analiquot consisting of 100µl of plant extracts (1µg/ml),1MNa2CO3 and Folin-Ciocalteau Reagent were added .The color developed after 15 minutes of there action in dark was measured at 760 nm against reagent blank and gallic acid was served as the reference standard .The total phenols were determined as mg of gallic acid equivalent per gm of sample using a calibration curve.

Estimation of total flavonoids
The total flavonoids present in plant extracts were determined with the colorimetric assay using $AlCl_3$ as published in literature (Aiyegoro *et al*., 2010) and performed in quintuplicate. To the appropriately diluted extracts (1ml), $AlCl_3$ (10%, 0.2ml), Potassium acetate (1M, 0.2ml) and distilled water (5.6ml) were added. The reaction mixture and incubated for 30 mi. at room temperature. The absorbance was recorded at 420 nm and the total flavonoids were determined using a calibration curve as mg of quercet in equivalent per gm of sample.

Estimation of Alkaloids
This was carried out as per the method of Harborne (1973). To 5 g of the sample, 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h and then filtered.The filtrate was concentrated on a wate rbath to one quarter of the original volume and subsequently added conc. NH4OH drop wise for the precipitation to occur. Then the precipitate was collected, washed with dilute NH4OH and then filtered. The residue thus obtained was dried and weighed.

Estimation of total Tannins
Tannin content was determined by using Folin Denis reagent (AOAC, 2005). Known aliquot of sample was added to a volumetric flask containing 75 ml of water in a 100 ml volumetric flask. Later 5 ml of Folin Denis reagent and 10 Na2CO3 solutions was added. After 30 minutes, the colour at 760 nm was measured against an experimental blank calibrated to 0 absorbency. Tannic acid percentage was determined by mg tannic acid from standard curve.

Estimation of total Terpenoids
This was carried out as per the method of Malik *et al*., 2017. The dried plant extract (100mg) was steeped in 9 ml ethanol for 24 hours and then filtered using Whatsmann filter paper. The filtrate was extracted with 10 ml of petroleum ether using a separating funnel . The ether extract was separated and dried completely in pre-weighed glass vials (final weight-wf). Ether was evaporated and the yield (%) of total terpenoids was calculated using the formula
W_i-W_f/W_i×100

Estimation of Saponins
The method used was that of Obdoni and Ochuko (2001) with slight modifications. To20gofsample, 100 ml of 20% aqueous ethanol was added, heated over a hot water bath for 2h with continuous stirring at about 55°C. The mixture was filtered and the residue was further extracted with 200 ml 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 25 0mL separation funnel and 20 mL of diethyl there was added and vortexes. The aqueous layer was recovered while

the ether layer was discarded. The purification process was repeated thrice and 60 mL of n- butanol was finally added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution evaporated, dried and the saponin content was determined.

Estimation of glycosides

This analysis was carried out according to Nbaeyi-Nwaoha and Onwuka (2014). Extracts (10g) were mixed with 10 mL of Baljet's reagent. After 1 h of incubation, 20mL of distilled water was added and the absorbance was measured at 495 nm. Securidaside was used as a standard ,and the amount of cardiac glycoside was expres sedas mg securida side equivalent(SE)/g.

Physiochemical Analysis

The air dried coarsely powdered leaves was subjected to physiochemical analysis such as determination of total ash, determination of water soluble ash, determination of acid in soluble ash, determination of sulphated ash, determination of loss on drying, determination of water soluble extractive value, determination of alcohol soluble extractive value and crude fibre content.

Determination of moisture content

AOAC method (1990) was followed for the estimation of moisture content. The extracts were set aside in hot air oven and drying was done at 105°C and the moisture was calculated as a measure of loss of weight. The crucible was set aside in oven and dried at 100°C for 30 min and weighed (W1). The finely powdered leaf sample (2g) were taken in crucibles and subsequently their weight was determined (W2). Then both samples and crucibles were kept in an oven and dried for 4h at 100 °C, cooled and weighed (W3). Subsequently, moisture content was determined by the following

$$\frac{(Initial\ weight\ of\ filled\ crucible)-(Final\ weight\ of\ filled\ crucible)}{(Initial\ weight\ of\ filled\ crucible)-(Initial\ weight\ of\ emty\ crucible)} \times 100\%$$

Determination of ash content

AOAC (1990) method was followed for determination of ash content. Finely powdered dried leaf sample (1g) were kept in muffle furnace and incinerated at 500 °C. The ash thus obtained was cooled and weighed and the ash content percentage was determined by the following

%Ash content=Weightofash /Weightoforiginal×100

Water soluble ash

The total ash was dissolved in 25ml water for 5 min and filtered and filter paper was subsequently transferred into crucible (silica), and kept in a muffle furnace for incineration at 450°C so that it becomes carbon free and weighed. The water-soluble ash percentage was calculated by using air-dried substance as a reference.

Acidinsolubleash

Tota lash was taken and dissolved in HCl (2N,25ml) for shaken for, filtered through filter paper (ashless). The filter paper was subsequently transferred into crucible (silica), and keptin a muffle furnace for incineration at 650°C so thatit becomes carbon free and weighed. The acid insoluble ash percentage was calculated and air-dried substance was employed as a standard.

Determination of crude fiber content

AOAC (1990) method was followed for this determination. First, the crucible was dried at 105°C and weighed after cooling. Sample (1g), filter agent (Celite 545 diatomaceous earth, 1g) was dissolved in sulfuric acid (0.25N, 200ml). The hydrolyzed mixture was filtered and the residue was washed with water toremove excess acid and filtered. Then NaOH (0.313 N, 200ml) was added and kept in boiling wate rbath for half an hour. The hydrolyzed mixture was filtered and the residue was washed with water to remove excess alkal iand filtered. Finally, it was rinsed in acetone and drained. Subsequently, the residue was driedin a crucible kept in oven at 105°C and then placed in the muffle furnace run at 550 °C (Meloan and Pomeranz 1980). Then the crucible was kept in desiccators and fiber content was determined as

(Weight of residue without ash) / (weight Sample) × 100 % = % Crude Fiber

Determination of alcohol soluble extractive value

The air-dried leaf powder (5g) was accurately weighed, macerated with ethyl alcohol (100ml) and shaken for 6h with final stand time of 18h. Then it was filtered and evaporated to dry nessina crucible and dried at 100°C. The ethanol soluble extractive value percentage (w/w) was calculated by employing the air-dried plant substance as a reference standard.

Determination of water-solubl eextractive value

The method was same as alcohol soluble extractive but chloroform and water in 1:300 was employed instead of ethanol.

Determination of nitrogen content

AOAC (1990) method was followed for determination of nitrogen. The Kjeldahl apparatus was steamed for about 10 min. After boric acid/indicator (5ml) was taken in a conical flask and positioned under the condenser. Then the digest (5ml) was taken into the apparatus and washed water, followed by NaOH (60%, 50ml) addition. Subsequently the digest was steamed and then $NH_4 (SO_4)_2$ was collected in a receiving flask. HCl (0.01M) was used for the treatment of receiving flask solution. A blank was also run simultaneously (James 1995) and the percentage of nitrogen was determined by

%Nitrogen= $(V_1-V_2)\times$ (molarity of the acid used) × 0.01410 × weight of sample ×100%

Where,

V_1=Volumeoftheacidusedfortesttitration

V_2 = Volume of acid used for blank titration

Determination of sulphated ash

The leaf powder (2g) were mixed with sulphuric acidin a crucible and made into a paste. The crucible was ignited gently until white fumes stop coming and placed in a desiccators for cooling purpose. The content was weighed and the sulphated ash percentage was determined with by using dried powdered plant substance as a standard.

III. RESULTS

The preliminary phytochemical analysis of ethanol and petroleum ether extracts of leaves of*G. amygdalinum* showed the occurrence of tannins, flavonoids, alkaloids,terpenoids, carbohydrates andsterols (Table-1). The total phenols accounts for 27.38 and 22.36 mg of gallic acid/gm respectively for ethanol and petether extracts. While the total flavonoids were 11.47and 9.25mg of quercetin/gm respectively for both the extracts (Table-2). The quantitative analysis of the phytochemicals was also carried out and the results were presented in table-3. The ethanol extract showed significantly higher phytochemical content compared to the pet ether extract. The alkaloids, tepenoids, tannins, glycosides and saponins were found be 13.28, 11.26, 7.12, 4.23 and 3.11 mg/100 g in the ethanol extract. While it was 11.47, 9.26, 5.32, 3.02 and 2.03 mg/100 g respectively in pet ether extract.

Table-1: Phytochemical composition extracts from leaves of *G.amygdalinum*

Partsused	Secondarymetabolites	EtOHextracts	Petetherextract
Leaves	Tannins	+	+
	Flavonoids	+	+
	Alkaloids	++	+
	Terpenoids	++	++
	Anthaquinones	-	-
	Glycosides	+	+
	Saponins	+	-
	Steroids	-	-

Table-2: Quantitative determination of the total phenols and flavonoids content

Extract	Totalphenolcontent (mg of gallic acid/g)	Totalflavonoidcontent (mg of quercetin/g)
Ethanolextract	27.38 ±1.01	11.47 ±0.98
Petether extract	22.36 ±1.09	9.25 ±0.79

Datapresented asMean± S.E(n=5)

Table-3: Quantitative determination of various phytochemicals in *G. amygdalinum*

S.No.	Phytochemicals	Pet Ether Extract	Ethanol Extract
		Amount (mg/100g)	Amount (mg/100g)
1.	Alkaloids	11.47±0.41	13.28±0.32
2.	Terpenoids	9.26±0.33	11.26±0.29
3.	Tannins	5.32±0.24	7.12±0.23
4.	Glycosides	3.02±0.16	4.23±0.17
5.	Saponins	2.03±0.09	3.11±0.12

Datapresented asMean± S.E(n=5)

Subsequently the physiochemical properties of leaves was also determined and presented (Table-2). The results of physicochemical properties indicate that leaves of *G. amygdalinum* have different content of moisture, total ash, acid and water insoluble ash, sulphated ash, alcohol soluble extractives and water-soluble extractives. The moisture content on dry basis was found to be 6.74%. The Ash contents were determined in a laboratory by subjecting the sample to burning at high temperature (removing the organic matter) and subsequently weighing the left out residue. The total ash,water soluble,acid in soluble and sulphated ash in leaves were measured to be 12.14%, 5.53%, 2.04% and 14.02% respectively.

Table-4:Physiochemical properties of extracts from leaves of *G. amygdalinum*

Properties	Percentage(%)
Totalash	12.14 ± 1.09
Watersolubleash	5.53 ± 0.77
Acidinsolubleash	2.04 ± 0.23
Sulphatedash	14.02 ± 1.17
Moisture	6.74 ± 0.44
Watersolubleextractivevalue	12.32 ± 1.04
Alcoholsoluble extractivevalue	24.01 ± 1.41
Crudefibre content	12.44 ± 1.36
Nitrogen	5.79 ± 0.82

Datapresented asMean± S.E(n=5)

IV. DISCUSSION

Alkaloids are well known phytoconstituents which have analgesic, antibacterial and antispasmodic properties (Harisaranraj *etal*., 2009;Uyo*etal*., 2013). Further, theyare usedinmedicine as anaesthetics andanalgesics (Herourat *etal*., 1988;Harborne,1988). Alkaloids has contributed to the majority of the poisons, neurotoxins and traditional psychedelics and social drugs (nicotine, caffeine, ephedrine cocaine and opiates) as reported (Zenk and Juenger,2007). Therefore,these beneficial properties of the alkaloids justify the traditional and modern medicinal usage of the investigated plant. The phenols and terpenes are well known important compounds in plants contributing to the antioxidant capacity of various botanicals (Kulkarni *et al*., 2010; Batool *et al*., 2019). Considerable research has been carriedout and established that flavonoids are antioxidants compounds and recognized as valuable nutraceuticals for neutralizing free radical stress (Batool *et al*., 2019). The presence of phenolics and flavonoids indicate that the botanical as a good contributor for its usage in traditional medicine to relieve from oxidative damage, stress and other related disorders. Flavonoids are known as excellent anti-oxidants which fight against liver tumors, toxins, allergies, inflammation, viruses and other microorganisms (Harisaranraj *et al*., 2009; Uyo *et al*., 2013). It has been well documented that these flavonoids delay cataract enlargement in diabetes (Haris aranra j*et al*., 2009). Tannins in minute quantities exertprominent anti-nutrient effect in diets as well as having some degree of astringency and can bind with proteins to form insoluble complexes, thus lessening protein bioavailability (Chikezie *et al*., 2008). Apart from this, these molecules possess antiviral, antibacterial, and antitumor properties (Kakiuch *et al*., 1986; Khanbabaea and Ree, 2000) and prescribed in the treatment of diarrhoea, dysentery and urinary tract infections (Fahey, 2005; Akinpelu and Onakaoya, 2009).

Glycosides have been known t opossess wide medicinal properties and employed in the treatment of congestive heart failure due to its diverse action which strengthens the force of myocardial contraction and action on smooth muscles as well (Braunwald *et al*., 1961). Its effects on neutral tissues and indirect effect on electrical activities of the heart and vascular resistance as well as capacitance has also been reported (Chukwuma *et al*., 2016). While saponins have the ability to coalesce with cholesterol, they impartabit tertaste and cause haemolytic activity in water solution (Sodipo*etal*.,2000) and have potential anti microbial properties (Sheikh*etal*.,2013). Anthraquinones are extensively studied phytochemicals due to thei rpotential applications in medicine. They possess antibacterial, anti-trypanosomal and antineoplastic activities (Velez and Osheroff, 2004; Heyman *et al*., 2009), anti- inflammatory and analgesic (Ayinde *et al*., 2007) and anti-oxidant properties (Okwu and Okwy, 2004).

The plant under investigation hosts all these phytoconstituents in appreciable amounts. Therefore, *G. amydalinum* plant could be explored as bio-friendly natural source of phytoconstituents, which serves as analternative to the conventional synthetic compounds for the treatment of various disorders.

V. CONCLUSION

The extracts from leaves showed significant amount of phytoconstituents such as alkaloids, flavonoids, phenolics, tannins, saponins and glycosides. The wide diversity and numerous secondary metabolites found in samples of this tree may be responsible for its copious medicinal and traditional usage. There is further scope of research in this area wherein, further are needed on various biochemical aspects of extracts where the data procured mighthelp in the development of lead products and suitable formulations for the treatment of various stress related disorders.

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