

1

2

3

4

5

6

7

8

9

Original Research Article **Phytochemical and mineral analysis of *Grewia mollis* stems bark**

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

ABSTRACT

Aims: Aqueous extract of *Grewia mollis* stem bark was analyzed for its phytochemical and mineral compositions.

Place and Duration of Study: Department of Biochemistry, University of Jos, Jos, between June 2013 and July 2013.

Methodology: The phytochemical screening and mineral composition of the stem bark was carried out using standard methods.

Results: The phytochemical screening revealed the presence of Tannins, Saponins, Cardiac glycosides, Flavonoids, Steroids, Phenols and Resins. Alkaloids and Carbohydrates were however not detected. Analysis of the mineral constituents showed that the plant contains among others Manganese (1.899 g/100g), Zinc (0.375 g/100g), Magnesium (287.060 g/100g), Iron (47.941 g/100g), Copper (0.270 g/100g) and Lead (0.206 g/100g).

Conclusion: The constituent phytochemicals and minerals may account for varied ethno-botanical uses of the plant in folk medicine.

Keywords: *Grewia mollis*; phytochemical constituents; Mineral constituents; Medicinal plants

30

31

32

33

34

35

36

1. INTRODUCTION

Plants that possess therapeutic properties or exert beneficial pharmacological effects in the animal body are generally termed as "medicinal plants". And It is now been established that the plants which naturally synthesize and accumulate some secondary metabolites and vitamins, possess medicinal properties [1]. Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents as well as important raw materials for manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role in the economy of a country [1]. Medicinal plants contain both organic and inorganic constituents, and many medicinal plants are found to be rich in one or more individual elements, thereby providing a possible link to the therapeutic action of the medicines [2, 3]. Trace elements play a vital role in the formation of bioactive chemical constituents in medicinal plants and are therefore responsible for both their medicinal and toxic properties [4]. Thus, a quantitative estimation of minor and trace element can be important in determining the effectiveness and toxicity of the medicinal plants in treating various diseases and understanding their pharmacological actions [5].

Grewia mollis (Tiliaceae) is a shrub or tree widely distributed in northern Nigeria and some African countries. It is known to be a strong fire-resistant plant and its various parts are used in food and medicines [6]. In Nigeria, the stem bark powder or mucilage of this plant is used as a thickener in local cakes made from beans and corn flour commonly called "*Kosa*" and "*Punkasau*" respectively [6,7]. The dried stem bark is ground and the powder mixed with beans or corn flour thereby enhancing the texture of the food product. Some findings demonstrated that mucilage obtained from the stem bark *G. mollis* can serve as a good binder

in paracetamol formulations [8, 9]. While as recent reports suggest that high concentration of this stem bark in dietary exposure may cause some adverse effects, especially liver injury [6]. The phytochemical studies of *G. mollis* indicated presence of tannins, saponins, flavonoids, glycosides, phenols and steroids but absence of alkaloids in the leaves and stem bark [10] while the presence of alkaloids was revealed in the roots [11]. The infusion of bark obtained by cold or hot maceration in water is applied to give a smooth surface to mud walls and floors [12] while the wood ash is used as salt substitute including the ash of the leaves, stems, and roots. Also, the flowers, buds, and young shoots are added to soups and sauces as garnishing while, in Sudan, the young leaves are cooked and eaten as vegetables. The fruit is eaten raw or boiled [13]. Studies have also demonstrated a wide spectrum of therapeutic effects such as hepatoprotective [14], antibacterial [15] and Anti-Inflammatory [15] activities. The present study was undertaken to evaluate the phytochemical and mineral compositions of *Grewia mollis* stem bark.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

The sodium hydroxide, ferric chloride, copper acetate, used were products of East Anglia Chemicals, Hadleigh Ipswich Suffolk; the petroleum ether (40 – 60°C) used was a product of Eagle Scientific Limited, Unit 5, Eldson Rd., Beeston, Nottingham; the chloroform, ammonium sulphate, sulphuric acid, naphthol, amyl alcohol, acetone, methanol, ethanol used were products of BDH Chemicals Ltd., Poole, England. Cupric sulphate, 5-hydrate was a product of J.T. Baker Chemicals Co., Phillipsmore, N.Z. All other reagents used were of analytical grade and prepared in the Biochemistry laboratory, University of Jos, Nigeria, with all-glass apparatus.

2.2 Plant Material

The stem bark of *G. mollis* was obtained from Akwanga-Nasarawa, Nasarawa State, Nigeria and was authenticated at Herbarium of the Department of Botany, University of Jos, Nigeria. A voucher specimen was deposited at the Herbarium of this Institute.

2.3 Preparation of Extract

The stem barks of *G. mollis* were washed and oven dried at 40°C for 72 hours to a constant weight. The dried pieces were then pulverized using an electric blender. The powdered material was stocked in a glass container from which 150 g was extracted in 1500 ml of cold distilled water for 48 hours at 37°C. This was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to give the extract. The extract was then used for phytochemical and mineral analysis.

2.4 Phytochemical Screening of the extract

The aqueous crude extract of *G. mollis* stem bark was subjected to various chemical tests in order to determine the secondary metabolites present by using the standard procedures as described by Sofowora [16], Trease and Evans [17] and Harborne [18]

2.4.1 Test for alkaloids

1ml of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide solution). The formation of a cream colour with Mayer's reagent indicates the presence of alkaloids.

2.4.2 Test for flavonoids

A few chop of 1% NH₃ solution was added to the aqueous extract of the plant sample in a test tube. A yellow coloration was observed for the presence of flavonoids.

2.4.3 Test for steroids

0.5 ml aqueous stem bark extract was evaporated and dissolved in 2 ml chloroform. 2 ml of conc. H₂SO₄ was introduced carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of steroid.

2.4.4 Test for phenols

5 ml of the extract was pipetted into a 30 ml test tube, and then 10ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added and left to react for 30 min. Development of bluish green colour was taken as a positive presence of phenol.

2.4.5 Test for resins

0.5 ml of extract was evaporated and dissolved in 2 ml of petroleum ether, 2ml of 2% copper acetate solution was then added and the mixture was shaken vigorously and allowed to separate, a green colour indicated the presence of resin.

2.4.6 Test for tannins

1ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue-black coloration which confirms the presence of tannin

2.4.7 Test for saponins

2 ml of double distilled water was added to 1 ml of the extract. Few drops of olive oil were added and agitated. Formation of soluble emulsion indicated the presence of saponin.

2.4.8 Test for cardiac glycosides

0.5 ml of the extract was evaporated and dissolved in 1 ml glacial acetic acid. One drop of 10% ferric chloride was then added. 1 ml of conc.H₂SO₄ was added by the side of the test tube. Appearance of brown colour ring at the interface indicated of presence of cardiac glycosides.

2.4.9 Test for carbohydrates

To 2 ml of the extract was added 2 drops of 10% alcoholic solution of naphthol followed by 2 ml concentrated H₂SO₄, gently poured along the side of the test tube at an angle of 45°. Formation of a purple ring at the interface of the two liquid layers confirmed a positive result for the presence of carbohydrates.

2.5 Mineral Analysis

The atomic Absorption spectrophotometer (AAS) was used for the analyses of Magnesium, Zinc, Iron, Manganese, Copper and Lead in the stem bark of *G. mollis*. Using AAS, a known amount of the sample was placed in a dish and heated with burner in a fume cupboard until there was no smoke emitted. This was transferred to the dessicator in other for it to cool after which 0.1 ml HCl solution was added to the ash. The resulting solution was filtered and diluted. Suitable salts of the metals in questions were used to make their standards, lamps were fixed and the analyses were done [19].

2.6 Statistical Analysis

The data were expressed as Mean ± Standard Error of Mean values.

3. RESULTS

Table 1 shows the phytochemicals present in *G. mollis* stem bark. Tannins, Saponins, Cardiac glycosides, Flavonoids, Steroids, Phenols, and Resins were detected in our study. However, Alkaloids and Carbohydrates were not detected which was in support with Onwuliri *et al.* [10].

The mineral analysis of *G. mollis* (Table 2) showed rich content of macro and micro elements like Manganese (1.899 g/100g), Zinc (0.375 g/100g), Magnesium (287.060 g/100g), Iron (47.941 g/100g) and Copper (0.270 g/100g). The plant also contains minute amount of Lead (0.206 g/100g).

Table 1: Qualitative phytochemical screening of *Grewia mollis* Stem Bark

Phytochemicals	<i>Grewia mollis</i> stem bark
Alkaloids	-
Flavonoids	+
Tannins	+
Saponins	++
Steroids	+
Cardiac glycosides	+
Phenols	+
Resins	+
Carbohydrates	-

Key= +: present; ++: highly present; -: absent

Table 2: Mineral composition of *Grewia mollis* Stem Bark

Minerals	Composition (g/100g)
Manganese	1.899±0.003
Zinc	0.375±0.001
Magnesium	287.060±0.001
Iron	47.941±0.002
Lead	0.206±0.001
Copper	0.270±0.001

Values are expressed as Mean ± SEM (n = 3).

DISCUSSION

A medicinal plant is any plant in which one or more of its organs contains substances that can be used for therapeutic purpose of which are precursors for the synthesis of useful drugs. The crude extracts or purified form of plant has been used as medicines and cosmetics [1]. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [20]. In this study, the phytochemical investigation of *G. mollis* stem bark indicates the presence of Tannins, Saponins, Cardiac glycosides, Flavonoids, Steroids, Phenols and Resins. This is in agreement with the work done by Onwuliri *et al.* [10]. These phytochemicals are well known for their pharmacological activities.

Saponins are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties [21]. According to Studies, saponins show antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. They seem to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability. Rao and Sung [22] found that saponins may help to prevent colon cancer. Anti-hyperglycemic potential of saponins has also been reported [23, 24]. Hence, due to the presence of saponin in *G. mollis* stem bark; it may be used use as an anti-diabetic agent. Furthermore, studies have shown that plants containing saponins have hemolytic activities on red blood cell membrane [25]. This may also be applicable to *G. mollis* stem bark.

177 Tannins and flavonoids are thought to be responsible for anti-diarrheal activity [26].
178 Investigations of their mode of action indicate that tannins and flavonoids increase colonic
179 water and electrolyte reabsorption while other phytochemicals act by inhibiting intestinal
180 mobility [26]. This explains the anti-diarrheal use of *G. mollis* stem bark in traditional medicine.
181 In addition, tannin has astringent properties, hastens the healing of wounds and inflamed
182 mucous membrane. Plants with tannins are used for healing of wounds, varicose ulcers,
183 hemorrhoids, frost-bite and burns [27, 28]. The biological functions of flavonoids include
184 protection against allergies, inflammation, platelets aggregation, microbes, ulcer and tumour
185 [29]. They are free radical scavengers, super antioxidants and potent water soluble thus
186 preventing oxidative damage and have strong anti-cancer activity [30]. Thus, the acclaimed
187 anti-inflammatory and wound healing uses of *G. mollis* stem bark extract may be attributed to
188 flavonoids and tannins. Cardiac glycosides were found to be present in *G. mollis* stem bark, a
189 compound that has been shown to aid in treatment for congestive heart failure and cardiac
190 arrhythmia. This is another reason why this plant is widely used in traditional medicine.
191 Cardiac glycosides work by inhibiting the Na^+/K^+ pump. This causes an increase in the level of
192 sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This
193 inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle,
194 improves cardiac output and reduces distention of the heart [31]. Phenolic compounds have
195 antimicrobial properties. Phenol and phenolic compounds have been extensively used in
196 disinfections and remain the standards which other bactericides are compared [32]. Thus,
197 the presence of phenolic compounds in *G. mollis* stem bark might be responsible for its
198 antimicrobial activities. Other phytochemicals such as Steroids and Resins might be
199 responsible for the some pharmacological activities of the plant.

200 Minerals which are either macrominerals or microminerals are essential for the normal growth
201 and maintenance of the body. The mineral analysis of *G. mollis* stem bark shows the
202 presence of Manganese, Zinc, Magnesium, Iron, Copper and minute amount of Lead (Table
203 2). Magnesium is involved in over 300 enzymatic reactions in the body [33, 34], including
204 glycolysis, the Krebs cycle, creatine phosphate formation, nucleic acid synthesis, amino acid
205 activation, cardiac and smooth muscle contractibility, cyclic AMP formation, and protein
206 synthesis. Magnesium is also important in the proper utilization of vitamins B and E and
207 functions with other minerals such as calcium, sodium and potassium in maintaining fluid and
208 electrolyte balance [35]. Copper is an essential trace element for humans and animals. The
209 ability of copper to easily accept and donate electrons explains its important role in oxidation-
210 reduction reactions and scavenging free radicals [36]. Iron is very important in the formation of
211 haemoglobin in red blood cells and deficiency of iron leads to anaemia. *G. mollis* stem bark
212 could be used as a source of iron in the treatment of iron-deficiency anaemia. Manganese
213 plays an important role in a number of physiologic processes, as a constituent of multiple
214 enzymes and an activator of other enzymes [37]. A number of manganese-activated enzymes
215 play important roles in the metabolism of carbohydrates, amino acids, and cholesterol. It is
216 also thought to aid in the maintenance of epithelia tissue [38]. Zinc is essential in the
217 activation of certain enzymes. These include dehydrogenase, alkaline phosphatase and
218 carboxypeptidase. Zinc containing organic compounds is employed as astringent and anti-
219 fungal agents. It aids wound healing and metabolism of nucleic acid and insulin. Zinc in
220 excess causes anaemia and if deficient in the body can lead to dermatitis [39]. Effect of lead
221 deficiency in the body results in anemia while excess lead (Pb) in the body can cause brain
222 damage [40].

223

224 5. CONCLUSION

225 This study shows that *G. mollis* stem bark is a rich source of significant phytochemicals and
226 minerals which justify the various therapeutic uses attributed to it in folklore medicine.
227 However the presence of trace amount of lead (Pb) calls for caution because of its toxicity.

228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280

REFERENCES

1. Sofowora A. Medicinal Plant and Medicine in Africa, John Willey Spectrum, Ibadan Nigeria. 1993;281:285.
2. Singh V, Garg AN. Availability of essential trace elements in Ayurvedic Indian medicinal herbs using instrumental neutron activation analysis. Appl Radiat Isot. 1997; 48(1):97-101
3. Ray DK, Nayak PK, Rautray TR, Vijayan V, Jena S. Elemental analysis of anti-diabetic medicinal plants using energy dispersive X-ray fluorescence technique. Indian J Phys. 2004;78B:103-105.
4. Rajurkar NS, Damame MM. Mineral content of medicinal plants used in treatment of diseases resulting from urinary tract disorders. Appl Radiat Isot. 1998;49(7):773-776.
5. Nafiu MO, Akanji MA, Yakubu MT. Phytochemical and Mineral Constituents of *Cochlospermum planchonii* (Hook. Ef. x Planch) Root. Bioresearch Bulletin. 2011;5:342-347
6. Obidah W, Godwin JL, Fate JZ, Madusolumuo MA. "Toxic effects of *Grewia mollis* stem bark in experimental rats. J Am Sci. 2010;6(12):1544-1548
7. Gill LS. Ethno-Medical Uses of Plants in Nigeria, University of Benin Press, Benin, Nigeria. 1992.
8. Martins E, Christiana I, Olobayo K. Effect of *Grewia* gum on the mechanical properties of paracetamol tablet formulations. Afr J Pharm Pharmacol. 2008;2:1-6.
9. Muazu J, Musa H, Musa KY. Compression, mechanical and release Properties of paracetamol tablets containing acid treated *Grewia* gum. J Pharmaceutical Sci Technol. 2009;1(2):74-79.
10. Onwuliri FC, Mawak JD, Wonang DL, Onwuliri EA. Phytochemical, toxicological and Histopathological studies of some medicinal plants in Nigeria. Int J Nat Appl Sci. 2006;2(3):225.
11. Heinz R, Helene F. The isolation of 6-Methoxyharmame from *Grewia mollis*, Lloydia. 1978;41(4):383.
12. Dalziel JM. The Useful Plants of West Africa, Crown Agents, London, UK. 1937.
13. Louppe D, Oteng-Amoako AA, Brink M. Plant Resources of Tropical Africa 7(1). Timber 1, PROVA Foundation, Wageningen, Netherlands; Backhuys Publishers, Lenden, Netherlands; CTA, Wageningen, Netherlands. 2008;298-300.
14. Asuku O, Atawodi SE, Onyike E. "Antioxidant, hepatoprotective, and ameliorative effects of methanolic extract of leaves of *Grewia mollis* Juss on carbon tetrachloride-treated albino rats. Journal of Medicinal Food. 2012;15(1):83-88.
15. Al-Youssef HMA, Amina M, El-Shafae AM. "Biological evaluation of constituents from *Grewia mollis*. J Chem Pharm Res. 2012;4(1):508.
16. Sofowora A. Medicinal Plants and Traditional Medicine in Africa (2nd Edition). Spectrum Books Ltd, Ibadan, Nigeria. 1993;p.289
17. Trease GE, Evans WC. Pharmacognosy, 11th edn. Bailliere Tindall, London. 1989;p.45-50.
18. Harborne JB. Phytochemical Methods. Chapman and Hall Ltd., London. 1973;p.49-188.
19. Oshodi AA. Proximate Consumption, Nutritionally valuable Minerals and Functional properties of *Adenopus breviflorus* benth seed and Protein concentrate. Food Chem. 1992;45:79-83.
20. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci Res Essay. 2007;2:163-166.
21. Prohp TP, Onoagbe IO. Determination of Phytochemical Composition of the Stem Bark of *Triplochiton Scleroxylon* K. Schum. (Sterculiaceae). IJABPT. 2012; p 69-75.
22. Rao AV, Sung MK. Saponins as anticarcinogens. J Nutri. 1995;125,717S-724S.
23. Malinow MR, McLaughlin P, Kohler GO, Livingstone AL. Alfalfa saponins: a family of substances potentially useful for treatment of hypercholesterolemia. J Clin Res. 1977;25:974-979.

- 281 24. Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus*
282 *sabdariffa*. J Med Plants Res. 2007;1(1):9-13.
- 283 25. Pathirana C, Gibney MJ, Taylor TG. Effects of soy protein and saponins on serum and
284 liver cholesterol in rats. Atherosclerosis. 1990;36,595-599.
- 285 26. Enzo AP. Traditional plants and herbal remedies used in the treatment of diarrheal
286 disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F,
287 Owais M, editors. Modern Phytomedicine: Turning Medicinal Plants in to Drugs. WILEY-
288 VCH Verlag GmbH & Co. KGaA, Weinheim. 2007;248-260.
- 289 27. Igboko DO. Phytochemical studies on *Garcinia Kola* Heckel. M. Sc Thesis. University of
290 Nigeria, Nsukka. 1983;202.
- 291 28. Maiduyi I. Biochemical and pharmacological studies of active principles of the seeds of
292 *Garcinia kola* Heckel. MSc. Thesis, University of Nigeria, Nsukka. 1983;p.108.
- 293 29. Okwu DE, Okwu ME. Chemical Composition of *Spondias mombin* Linn plant part.
294 J.Sustain. Agric. Environ. 2004;6(2):140-147.
- 295 30. Salah W, Miller N, Pagauga G, Tybury G, Bolwell E, Rice E, Evans C. Polyphenolic
296 flavonoids as scavenger of aqueous phase radicals and chain breaking antioxidants. Arch
297 Biochem. 1995;2:239-346.
- 298 31. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six
299 Malaysian medicinal plants. *Journal of Medicinal Plants Research*. 2009;3(2):67-72
- 300 32. Okwu DE. Evaluation of Chemical composition of indigenous species and flavouring
301 agents. Global J Pure Appl Sci. 2001;p.458-459.
- 302 33. Wester P. Magnesium. Am J Clin Nutr. 1987;45:1305-1312.
- 303 34. Berdanier CD. In: Advanced Nutrition-Micronutrients. CRC Press, New York. 1994;23-34.
- 304 35. Bakhru HK. In: Vitamins that heal. Orient Paper Backs Delhi, India. 1999;12.
- 305 36. Linder MC, Hazegh-Azam M. Copper Biochemistry and Molecular Biology. *Am J Clin Nutr*.
306 1996;63(5):797S-811S.
- 307 37. Nielsen FH. Ultratrace minerals. In: Shils M, Olson JA, Shike M, Ross AC. eds. Modern
308 Nutrition in Health and Disease. 9th ed. Baltimore: Williams & Wilkins. 1999;283-303.
- 309 38. Martin DW, Mayers PA, Rodwell VW, Granner DK. Harper's Review of Biochemistry, 20th
310 ed. Lange Medical Publications, California. 1985;651-660.
- 311 39. Akpabio UD, Ikpe EE. Proximate composition and nutrient analysis of *Aneilema*
312 *aequinoctiale* leaves. Asian Journal of Plant Science and Research. 2013;3(2):55-61
- 313 40. Stoker SH. Environmental Chemistry: air and water pollution. 2nd ed., Wiley Interscience
314 Publishers, U.S.A. 1976;2-29.