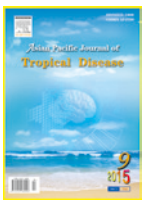
HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Original article doi: 10.1016/S2222-1808(15)60918-5

Free-radical scavenging activity and bioactive secondary metabolites from various extracts of *Glinus oppositifolius* (L.) Aug. DC. (Molluginaceae) roots, stems and leaves

Juliana Janet R. Martin-Puzon*, Windell L. Rivera

Institute of Biology & Natural Sciences Research Institute, University of the Philippines Diliman, Quezon City 1101, Philippines

ARTICLE INFO

Article history:
Received 22 Apr 2015
Received in revised form 6 May 2015
Accepted 30 Jun 2015
Available online 15 Jul 2015

Keywords:
Glinus oppositifolius
In vitro antioxidant
Plant extracts
Phytochemicals
Secondary metabolites
Bioactive compounds

ABSTRACT

Objective: To evaluate the *in vitro* antioxidant activity of *Glinus oppositifolius* (*G. oppositifolius*) and to determine the presence of phytochemical constituents in ethanol, methanol and chloroform extracts of the roots, stems and leaves of *G. oppositifolius*.
Methods: The extracts were examined for antioxidant property by free–radical [2,2–diphenyl–1–picrylhydrazyl (DPPH)] scavenging activity and screened for the presence of bioactive phytochemical constituents, *i.e.*, alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and terpenes. The total phenolic and total flavonoid contents were quantified by Folin–Ciocalteu method and aluminum chloride method, respectively.
Results: The results showed that all the plant parts, *i.e.*, roots, stems and leaves, exhibited free–radical scavenging activity which can be attributed to the presence of phytochemical constituents in the various extracts. The root chloroformic extract had the highest DPPH inhibition activity which was 70% relative to gallic acid, followed by the root methanolic and ethanolic extracts exhibiting 37% and 28% DPPH inhibition activity, respectively.
Conclusions: This paper has reported for the first time the antioxidant activity of the different parts of *G. oppositifolius*, *i.e.*, roots and stems. The results demonstrate great potential of the plant as a new source of food supplements, drug components and other materials or ingredients for health and wellness.

1. Introduction

Antioxidants are compounds which have the ability to scavenge or trap free radicals. Studies on finding antioxidant phytochemicals are significant because those phytochemicals can inhibit the propagation of free–radical reactions and protect the human body from metabolic diseases due to oxidative stress such as DNA damage, carcinogenesis and degenerative disorders like cardiovascular diseases, aging and neurodegenerative diseases, atherosclerosis and rheumatoid arthritis. Free radicals include the reactive oxygen species, namely, superoxide anion, hydrogen peroxide, peroxyl radicals and nitric oxide[1]. Antioxidants are compounds which inhibit oxidation or free radical–induced oxidative damage and thus are potential quenchers of oxidative stress–induced lipid peroxidation[2].

The use of synthetic antioxidants has been restricted due to their side effects, such as inflammation and carcinogenicity. Therefore, the interest and importance of the search and exploitation of naturally occurring antioxidants in medicinal and edible plants to replace synthetic antioxidants have tremendously increased in recent decades[1,2]. Many studies revealed a vast number of plants with antioxidant capabilities. However, the antioxidant potential of the different parts of *Glinus oppositifolius* (*G. oppositifolius*) remained unexploited.

Plants are rich sources of bioactive substances, such as alkaloids, flavonoids, glycosides, saponins, sterols, tannins, terpenes, and other metabolites with antioxidant activity. Various reports have shown that many of these phytochemical compounds possess antibacterial, antifungal, antiviral, antiprotozoal, antihelminthic, antidiarrhoeal, anticarcinogenic,

*Corresponding author: Juliana Janet Martin-Puzon, Institute of Biology & Natural Sciences Research Institute, University of the Philippines Diliman, Quezon City 1101, Philippines.
Tel: (632) 920-5471
E-mail: janetmpuzon@gmail.com
Foundation Project: Supported by Bureau of Agricultural Research, Department of Agriculture of the Philippines for the research funds through the Postdoctoral Research Fellowship Grant for Basic Research in Agriculture and Fisheries of Juliana Janet R. Martin-Puzon.

anti-inflammatory, antiartherosclerotic and antidiabetic activities.

Commonly known in Philippines as "sarsalida" (Tagalog), "papait" (Iloko) and slender carpet weed (English), *G. oppositifolius* [syn. *Mollugo oppositifolia* and *Mollugo spergula* (L.)] belongs to the family Molluginaceae[3]. The plant is a very common weed that grows at low and medium altitudes throughout Philippines. It also occurs in India, tropical Africa and Australia. The plant is a slender or ascending, smooth, branched, annual herb, with branches 10–40 cm in length. The leaves are opposite or whorled, spatulate, oblanceolate to oblong–obovate, 1–3 cm long, and up to 1 cm wide. The flowers are white and fascicled, with slender stalks up to 1 cm long, sepals 33–50 mm long and ellipsoid capsule which is a little shorter than the sepals. The seeds are numerous and covered with raised tubular points.

G. oppositifolius has a lot of therapeutic values in traditional medicine. It is reported to have analgesic, antidiabetic, anti-hyperlipidemic, antihelminthic, antidiarrhoeal, diuretic, antimalarial, antiviral, antimicrobial and antioxidant properties[4,5]. The shoot of *G. oppositifolius* is eaten occasionally as a vegetable, even though it is bitter, on account of its stomachic, aperient, and antiseptic properties[3,4]. The whole plant, without the roots, is used as a cooked cataplasm for treatment of dyspepsia in children and as an infusion to promote the menstrual discharge in women. It is used as a blood purifier and liver stimulant. It can also improve digestion and can cure burning sensation, itchiness and other skin ailments. This study is the first report aimed to evaluate the *in vitro* antioxidant activity and to determine the presence of phytochemical constituents in ethanol, methanol and chloroform extracts from the roots, stems and leaves of *G. oppositifolius*.

2. Materials and methods

2.1. Plant samples

The whole plant of *G. oppositifolius* was collected from Barrio Colibangbang, Paniqui, Tarlac, Philippines in February and March 2014. The taxonomic identification of the plant was authenticated by a curator in the Botany Division of the National Museum, Manila where a voucher specimen was also deposited, with control No. 255-2014.

2.2. Drying and extraction

In the laboratory, collected plant materials were washed thoroughly in running tap water to remove soil particles and other debris, and shade-dried in an air-conditioned laboratory for 2–3 weeks. While being dried, the different plant parts were segregated into roots, stems and leaves. The segregated roots, stems and leaves were separately powdered by a mechanical grinder and then stored in airtight closed containers before used for analysis. One hundred grams of the different parts of *G. oppositifolius* were added separately to 500 mL ethanol, methanol and chloroform, and soaked for 3 days. Removal of the plant residue from each of the solvents was done by filtration and the resulted filtrate was concentrated under reduced pressure at 40 ℃ on a rotary evaporator (Laborota 4001, Heidolph). The concentrated filtrates were transferred into Petri dishes and allowed to air-dry completely.

2.3. 2,2–Diphenyl–1–picrylhydrazyl (DPPH) assay

The DPPH free–radical scavenging assay was performed to determine the antioxidant activity of the various extracts by using gallic acid as the positive control and dimethylsulfoxide (DMSO), the solvent of the extracts, as the negative control[6]. Five microliters of the controls and extracts were added to make a final volume of 100 μL. Four milligrams of each of the extracts and controls were used in the assay. After incubation at 37 ℃ and 5% CO_2 for 45 min, absorbance was read at 570 nm. Based on the absorbance readings, free radical inhibition of the plant extracts was computed by using the formula:

Inhibition (%) = [(Absorbance of DMSO - Absorbance of extract) / (Absorbance of DMSO - Absorbance of gallic acid)] × 100

Three trials from two separate batches of plant extracts were done in triplicates.

2.4. Phytochemical screening

The leaf, stem and root extracts obtained from ethanol, methanol and chloroform extraction solvents were analyzed for the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins and triterpenes according to standard methods[7,8].

2.5. Estimation of total phenolics and total flavonoids

The total phenolic content of each of the methanolic extracts was determined by the modified Folin–Ciocalteu method[1]. Dried methanolic extracts from the roots, stems and leaves were redissolved separately in methanol to make a final concentration of 1 mg/mL. About 50 μL of Folin–Ciocalteu reagent and 100 μL of Na_2CO_3 (2%, w/v) were added to 50 μL of plant extract solution (1 mg/mL). The resulted mixture was incubated at 45 ℃ with shaking at 120 r/min for 15 min. The absorbance of the samples was measured at 620 nm, with a plate reader. Results were

expressed as μg gallic acid equivalents/ mg plant extract. The same procedure was followed to make standard curve by using gallic acid as standard and methanol at concentration range of 0–100 μg/mL. Each of the plant extract solutions was subjected to total phenolics content determination in triplicates.

The flavonoids content of each of the methanolic extracts from the leaves, stems and roots was determined by using the aluminum chloride method with some modifications[1]. Dry methanolic extracts from the roots, stems and leaves were redissolved separately in methanol to make a final concentration of 1 mg/mL. About 100 μL sample (1 mg/mL) was mixed with 300 μL methanol, 20 μL of 10% aluminum chloride, 20 μL of 1 mol/L potassium acetate and 560 μL of distilled water, and kept at room temperature for 30 min. The absorbance of the samples was measured at 405 nm by using a plate reader. The total flavonoid content of the plant extracts was determined from calibration curve made by rutin as standard and methanol at a concentration range of 0–100 μg/mL. The concentration of total flavonoids was expressed as μg rutin equivalents/ mg plant extracts.

2.6. Statistical analysis

Data on free–radical scavenging activity (%), and total phenolics and flavonoid contents were analyzed by Kruskal–Wallis test and Tukey's HSD *post hoc* analysis by using SPSS 7. These data were expressed as mean ± SD of triplicates.

3. Results

The yield (%) of extracts using the different solvents is shown in Table 1. Compared with the different plant parts, the leaves consistently had the highest yield, regardless of the extraction solvent used. The highest yields were obtained from leaves extracted with chloroform and methanol. Low yields were obtained from roots extracted with ethanol and chloroform, as well as stems extracted with chloroform.

Table 1
Yield of extracts obtained from different dried parts of *G. oppositifolius* with different solvents. %.

Plant parts	Ethanol	Methanol	Chloroform
Leaves	3.63	8.47	9.32
Stems	3.09	7.85	1.24
Roots	1.06	2.93	1.31

The antioxidant activity of the segregated dried leaves, stems and roots of *G. oppositifolius* extracted with different solvents, *viz.*, 95% ethanol, 99% methanol and chloroform, was examined through the DPPH free–radical scavenging assay. The determination was conducted with triplicates per extract. Gallic acid served as the positive control while DMSO, the solvent for the extracts used in the assay served as the negative control.

The mean percentage of DPPH inhibition activity of *G. oppositifolius* extracts from leaves, stems and roots obtained from two independent extractions are reflected in Table 2. All extracts from the different parts of *G. oppositifolius* exhibited DPPH inhibition activity ranging from 3.503%-69.957%, relative to gallic acid with 100% inhibition and DMSO with 0% inhibition. The root chloroformic extract, root methanolic extract and root ethanolic extract exhibited the top three highest DPPH inhibition activity.

Table 2
Free–radical inhibition activity of leaf, stem and root extracts of *G. oppositifolius* by using DPPH antioxidant assay. %.

Plant organs	Ethanol extract	Methanol extract	Chloroform extract
Leaves	$18.006 \pm 2.720^{c,d}$	13.747 ± 2.170^{b}	$18.987 \pm 4.450^{b,c}$
Stems	$23.219 \pm 1.540^{c,d}$	3.503 ± 0.490^{a}	12.802 ± 2.160^{b}
Roots	27.594 ± 3.310^{d}	37.143 ± 1.010^{e}	69.957 ± 4.860^{f}

Data are means ± SD from two separate extractions with triplicates per extract. Data with different superscript letters are significantly different from each other.

The phytochemical analysis showed that all the plant parts contained all the secondary metabolites examined (Table 3). The most diverse classes of secondary metabolites were present in the leaf ethanolic extract. Specifically, this extract contained alkaloids, flavonoids, glycosides, saponins, sterols and tannins; however, no triterpenes were detected. Leaf methanolic extract, and stem and root ethanolic extracts contained five of the metabolite classes tested. Specifically, flavonoids, glycosides, saponins, sterols and tannins were present in the leaf methanolic extract while alkaloids and triterpenes were not detected. Stem ethanolic extract contained all the metabolites tested, except for sterols and tannins. Moreover, flavonoids and tannins were absent in root ethanolic extracts while the other five metabolite classes were present. The other extracts, *i.e.* leaf chloroformic extract, as well as stem and root methanolic and chloroformic extracts, contained four classes of secondary metabolites detected in this study.

The total phenolics content of methanolic extracts from the leaves, stems and roots was (38.52 ± 1.08), (26.30 ± 1.03) and (27.97 ± 1.20) μg gallic acid equivalent/ mg dried plant extracts, respectively, with reference to gallic acid standard curve (y = 0.004 8x + 0.011 9, R^2 = 0.987 7). The total flavonoid content of methanolic extracts from the leaves, stems and roots was (86.47 ± 4.24), (25.88 ± 3.11) and (39.61 ± 2.78) μg rutin equivalent/ mg plant extracts, respectively, with reference to rutin standard curve (y = 0.001x + 0.051, R^2 = 0.994). The phenolics, including flavonoids and other phytochemical compounds present in the leaves, stems and roots of *G. oppositifolius* are bioactive compounds that play a role in antioxidant activities of the plant extracts.

Table 3

Phytochemical screening of secondary metabolite constituents in crude ethanolic, methanolic and chloroformic extracts from different parts of *G. oppositifolius*.

Secondary metabolites	Phytochemical tests	Leaf extracts			Stem extracts			Root extracts		
		Ethanol	Methanol	Chloroform	Ethanol	Methanol	Chloroform	Ethanol	Methanol	Chloroform
Alkaloids	Mayer's test	+	-	+	+	-	+	+	+	+
	Wagner's test	+	-	+	+	-	+	+	+	+
Flavonoids	Shinoda's test	+	+	-	+	+	-	-	-	-
Glycosides	Fehling's test	+	+	+	+	+	+	+	+	+
Saponins	Froth test	+	+	+	+	+	+	+	+	+
	Sulfuric acid test	+	+	+	+	+	+	+	+	+
	Frohde test	+	+	+	+	+	+	+	+	+
Sterols	Libermann–Burchard test	+	+	+	-	-	+	+	-	+
Tannins	Ferric chloride test	+	+	-	-	-	-	-	-	-
	Gelatin-salt black test	+	+	-	-	-	-	-	-	-
Triterpenes	Libermann–Burchard test	-	-	-	+	+	-	+	+	-
	Salkowski's test	-	-	-	+	+	-	+	+	-

+: Presence; -: Absence.

4. Discussion

The free–radical scavenging activity of the different extracts was examined by their ability to reduce the 1,1-diphenyl-2-picrylhydrazyl, a stable free radical which can accept an electron or hydrogen from the plant metabolites. DPPH is a purple-colored dye with maximum absorption at 517 nm; and when the antioxidants present in the plant extracts scavenge free radicals by hydrogen donation, its purple color fades or disappears due to its conversion to 2,2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance[6].

The antioxidant potentials of the different extracts were found to be in the following order: root chloroformic extract > root methanolic extract > root ethanolic extract ⩾ stem ethanolic extract = leaf ethanolic extract ⩾ leaf chloroformic extract ⩾ leaf methanolic extract = stem chloroformic extract > stem methanolic extract. Based on the results of the DPPH assay, it is conclusive that the roots exhibited the greatest antioxidant potential, compared to the shoots or above-ground organs. The difference in the antioxidant activities between the leaves and stems is probably due to the extraction procedures and sample processing which possibly result in the destruction or evaporation of some compounds[9]. However, the difference in the secondary metabolite constituents of the two plant organs can be explained by the difference in antioxidant activity as reflected in the results of the phytochemical analysis.

The antioxidant activity of the leaves, stems and roots can be attributed to the presence of various phytochemicals detected in the extracts. The antioxidant property of the extracts is one of the most important contributing factors for the applications of the plants in the management and treatment of various diseases caused by oxidative stress[10]. They prevent the cells and vital biomolecules from damage caused by free radicals by terminating chain reactions triggered by highly reactive free radicals, removing free radical intermediates and inhibiting other oxidation reactions[11]. Phenolic compounds, also known as polyphenols or phenylpropanoids, are a large family of secondary metabolites derived from the aromatic amino acids. This diverse group of secondary metabolites range from a simple phenolic acids to very large and complex polymers, such as tannins, lignans and lignins, including flavonoids[12].

Flavonoids and other phenolic compounds have been reported to be associated with antioxidant activity, mainly due to their redox properties, which play a role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[13]. Antioxidant property of phenols, especially flavonoids, is valuable for therapeutic and prophylactic applications after infection, inflammation, burns or radiation injury. Moreover, tannins also serve as antioxidants which are 15–30 times more effective in quenching peroxyl radicals compared to simple phenolics[13].

Phytochemicals or secondary metabolites in plant samples are known to be biologically active compounds and they are responsible for different activities, such as antioxidant activity[9]. The different extracts obtained by using ethanol, methanol and chloroform as solvents were screened for the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins and triterpenes with standard phytochemical tests (Table 3). The medicinal value of plants lies in the different phytochemicals that have been found to possess a wide range of activities or definite physiological action on the human body, which may help in protection against chronic diseases[14]. Alkaloids have a bitter taste and have been associated with medicinal uses for centuries and one of their common biological properties is their toxicity[15]. There have been several reports on their analgesic, antispasmodic and antibacterial properties. Aside from the antioxidant activity, flavonoids exhibit a wide range of biological activities like antimicrobial, anticancer, anti–inflammatory, anti–allergic, and analgesic properties, among others[9]. Glycosides are condensation products of sugars, including polysaccharides with different varieties of organic hydroxyl (occasionally thiol) compounds (invariably monohydrate), in such a manner that the hemiacetal entity of the carbohydrate must take part in the condensation[16]. These compounds possess intensely bitter principles containing the lactone group that may be diterpene lactones or triterpenoids. Some of the bitter principles are used either as astringents due to the presence of tannic acid, or as antiprotozoa. Examples are cardiac glycosides which act on the heart, anthracene glycosides which serve as purgative and for treatment of skin diseases, and chalcone glycoside which is anticancer. Saponins are terpene glycosides with detergent properties[12]. They demonstrate hemolytic activity, cholesterol–binding properties and bitterness[15].

They exhibit antidiarrhoeal, anticancer and antihelminthic activities[17].

Steroidal compounds have antibacterial and antiviral properties[18]. They are also important due to their relationship with various anabolic hormones including the sex hormones[15]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers[9]. They form complexes with proteins, carbohydrates, gelatins and alkaloids. Tannins are divided into hydrolysable tannins and condensed tannins[16]. Upon hydrolysis, hydrolysable tannins produce gallic acid and ellagic acid depending on the type of acid produced. Tannins possess anti–inflammatory, antiseptic, antimicrobial, anticancer, antidiarrhoeal and antidysentrial activities[18,19]. In *G. oppositifolius*, tannins were found only in the leaf extracts. The triterpenes constitute another group of secondary metabolite which has antioxidant, anti–inflammatory, sedative, insecticidal and cytotoxic activities. Common triterpenes are major components of essential oils[16]. A triterpenoid saponin in *G. oppositifolius* was reported to exhibit antidiabetic property[20].

In conclusion, phytochemical screening revealed that the entire *G. oppositifolius* plant, *i.e.*, roots, stems and leaves (including flowers) was a rich source of alkaloids, flavonoids, glycosides, saponins, sterols, tannins and triterpenes. The various biological activities of *G. oppositifolius*, including its antioxidant activity, are due to the presence of diverse secondary metabolites. The presence of phytochemical compounds in *G. oppositifolius* makes the plant useful for the treatment of many ailments as previously reported. It strongly indicates its potential as a source of drugs, drug components, nutriceuticals, food supplements, and other materials or ingredients for health and wellness.

Research work is underway towards the isolation, identification, purification, and characterization of the constituents responsible for the various biological activities of this plant by using chromatographic and spectroscopic analyses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We thank the Bureau of Agricultural Research, Department of Agriculture of the Philippines for the research funds through the Postdoctoral Research Fellowship Grant for Basic Research in Agriculture and Fisheries of Juliana Janet R. Martin-Puzon from 1st February 2014 to 30th April 2015; and we thank the Natural Sciences Research Institute, University of the Philippines Diliman, Quezon City for hosting the research work.

References

[1] Rameshkumar A, Sivasudha T. *In vitro* antioxidant and antibacterial activity of aqueous and methanolic extract of *Mollugo nudicaulis* Lam. leaves. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S895-900.

[2] Nagesh KS, Shanthamma C. Micropropagation and antioxidant activity of *Mollugo nudicaulis* Lam. *J Med Plants Res* 2011; **5**(6): 895-902.

[3] Quisumbing E. *Medicinal plants of the Philippines*. Quezon City: Katha Publishing Co., Inc.; 1978.

[4] Pattanayak S, Nayak SS, Dinda SC, Panda D. Preliminary anti-diarrhoeal activity of aerial parts of *Glinus oppositifolius* (L.) in rodents. *Recent Adv Pharm Sci Res* 2012; **1**(2): 50-7.

[5] Pattanayak S, Nayak SS, Dinda SC, Panda D, Kolhe DM. Antimicrobial and anthelmintic potential of *Glinus oppositifolius* (Linn.) family: Molluginaceae. *Pharmacologyonline* 2011; **1**: 165-9.

[6] Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol* 2004; **26**(2): 211-9.

[7] Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis.* London: Chapman and Hall; 1988.

[8] Evans WC. *Trease and Evan's pharmacognosy.* 15th ed. Edinburgh: WB Saunders; 2002.

[9] Alabri THA, Al Musalami AHS, Hossain MA, Weli AM, Al-Riyami Q. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *J King Saud Univ Sci* 2014; **26**(3): 237-43.

[10] Agbafor KN, Nwachukwu N. Phytochemical analysis and antioxidant property of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. *Biochem Res Int* 2011; doi: 10.1155/2011/459839.

[11] Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol* 1997; **82**(2): 291-5.

[12] Hopkins WG, Huner NPA. Secondary metabolites. In: Hopkins WG, Huner NPA, editors. *Introduction to plant physiology*. 4th ed. New York: John Wiley & Sons, Inc.; 2009, p. 459-80.

[13] Mašković PZ, Manojlović NT, Mandić AI, Mišan AČ, Milovanović IL, Radojković MM, et al. Phytochemical screening and biological activity of extracts of plant species *Halacsya sendtneri* (Boiss.) Dörfl. *Hemijska Ind* 2012; **66**(1): 43-51.

[14] Mir MA, Sawhney SS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker J Pharm Pharmacol* 2013; **2**(1): 1-5.

[15] Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. *J Phytol* 2011; **3**(12): 10-4.

[16] Doughari JH. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: Venketeshwer Rao, editor. *Phytochemicals-a global perspective of their role in nutrition and health*. Rijeka: Intech; 2012.

[17] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Int Pharm Sci* 2011; **1**(1): 98-106.

[18] Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr J Pharm Pharmacol* 2009; **3**(2): 58-62.

[19] Kalimuthu K, Prabakaran R. Preliminary phytochemical screening and GC-MS analysis of methanol extract of *Ceropegia pusilla*. *Impact Int J Res Appl Nat Soc Sci* 2013; **1**(3): 49-58.

[20] Kumar D, Shah V, Ghosh R, Pal BC. A new triterpenoid saponin from *Glinus oppositifolius* with α-glucosidase inhibitory activity. *Nat Prod Res* 2013; **27**(7): 624-9.