European Journal of Medicinal Plants

28(1): 1-9, 2019; Article no.EJMP.30580
ISSN: 2231-0894, NLM ID: 101583475

Comparative Analysis of Bioactive Compounds in Two Selected Plants *(Verononia amygdalina* and *Jatropha gossypifolia)*

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Authors' contributions

This work was carried out in collaboration between both authors. Author OAJ designed the study. Author AOI performed the statistical analysis. Author OAJ wrote the protocol and wrote the first draft of the manuscript. Authors AOI and OAJ managed the analyses of the study. Authors AOI and OAJ managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2019/v28i130124
Editor(s):
(1) Dr. Shanfa Lu, Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Bouyahya Abdelhakim, Mohamed V University, Morocco.
(2) Taiwo O. Elufioye, University of Ibadan, Nigeria.
(3) Sachin K. Jain, Pharmacognosy IPS Academy Collge of Pharmacy, Indore RGT University Bhopal, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/30580

Original Research Article

Received 29 January 2017
Accepted 20 April 2017
Published 10 May 2019

ABSTRACT

Chemical analysis were carried out to investigate the bioactive constituents of *Vernonia amygdalina* and *Jatropha gossypifolia* and to determine the quality of saponin, alkaloid, and tannin contents of the selected plants. In comparing the bioactive constituents of *Vernonia amygdalina* and *Jatropha gossypifolia*, the results showed that there were significant presence of alkaloids, streoids, flavonoids, phenolic compounds, condensed tannins, glycosides and reducing sugar in both plants while some were absent such as cardiac glycosides, hydrolysable tannin, phlobatannin, terpenoids, polysaccharide/starch and coumarin. The result also revealed the concentration of some of the bioactive compounds such as saponins 1.103 g/dm^3 in *Vernonia amygdalina* compare with 1.079 g/dm^3 in *Jatropha gossypifolia*. Tannins 26.48 g/dm^3 in *Vernonia amygdalina*, 24.12 g/dm^3 in *Jatropha gossypifolia* and alkaloids; 0.580 g/dm^3 in *Vernonia amygdalina*, 0.7585 g/dm^3 in *Jatropha*

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gossypifolia. The bioactive compounds detected in these plants are of therapeutic importance and their presence indicates the beneficial effects of the plants and also supports the use of these plants in ethno-medicine for the management of various ailments.

Keywords: V. amygdalina; J. gossypifolia; phytochemicals.

1. INTRODUCTION

Food such as carbohydrates, proteins, and fats and oils are consumed by man and animals. Other chemical compounds in plants apart from these are phytochemicals. Such compounds usually exert unique and specific active phytochemical effects responsible for their therapeutic and pharmacological functions [1]. Activities of such naturally occurring compounds are generally responsible for changes, which are used to satisfy man's desires. They do not add to body calorie and are numerous in types. They are applied mostly for preventive and healing purposes [2].

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Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life [3]. Phytochemicals also known as phytonutrients and naturally occurring substances found in plants [4]. These substances have been found to be beneficial to human health as well as possessing antioxidant activity [5]. Many common plants based foods and herbs contain powerful phytochemical substances that can improve the quality of health. Phytochemicals can also protect us against many diet related diseases. Phytochemicals could act as an antioxidant and anti-inflammatory. It plays vital role in detoxification of harmful and deleterious chemicals of the body. The term phytochemicals refers to a wide variety of compounds made by plants, but is mainly used to describe those compounds that may affect human health. Phytochemicals are found in plant-based food such as fruits, vegetables, beans and grains. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely. Some known phytochemicals include beta carotene and other carotenoids, ascorbic acid(vitamin C), folic acid and vitamin E. it is well known that plant produce these chemicals to protect themselves but recent research demonstrate that they can also protect human against diseases.

Jatropha gossypifolia is a perennial herb from the Euphorbiaceae family. Jatropha grows in tropical and sub- tropical region with cultivation limits at 30ºN and 35ºS it also grows in lower latitude of 0-500meters above sea level. It may flower at any time of the year. The cultivation of jatropha may either be by cutting which is an asexual means or by seed which is the sexual means of propergation. It forms a small, spreading shrub with a sparse, open canopy reaching to 1m in height. It releases a sticky, yellow translucent sap when injured. The leaves are alternate, 10 cm wide with hairy margins and are deeply divided into 3 to 5-pelated in small, terminal clusters and are deep rich maroon in colour. The fruits are 3-lobed, mature; the dry fruit is seldom seen because it splits open explosively when dry, scattering the 3 enclosed seed in all direction [6]. The leaves and seeds of *Jatropha gossypifolia* is considered a purgative and is widely used to treat obstinate constipation.

Vernonia amygadalina is a genus of about 1000 species of forbs and shrubs in the family asteraceae. Some species are known as iron weed, some are edible and of economic value. These are known for having intense purple flowers. The genus is named for English botanist William Veron. There are numerous distinct subgenera and subsections in this genus. Several species of vernonia, including *V. calvoana, V. amygdalina and V. colorata,* are eaten as leaf vegetables. Common names for these species include bitter leaf, (ewuro local Yoruba name). Some of the health benefits of *V. amygdalina* include speeding up of metabolism and eventual weight loss, relieving fever and feverish conditions, reduces high sugar level in the blood and as such great for diabetic patients, soothes and cures pile, the leave juice nourishes the skin and also cures mild stomach ailments [7]. *V. amygdalina* can be found in drainage lines and in natural forests or at home and commercial plantations [8]. *V. amygdalina is usually* propagated by cuttings [9] but studies found that bee infested flowers would be formed under drastic growth environment and the seeds from these flowers could then thrived well in slightly acidic soil [10]. *V. amygdalina* is well known as a medicinal plant with several uses attributed to it including diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache and joint pain

associated with AIDS, hence the need to investigate the presence of bioactive compounds present in them.

2. MATERIALS AND METHODS

2.1 Materials

Soxhlet extractor, flat bottom flask, heating apparatus, retort stand, spatula, thimble, tube, water bath, test tubes, beakers, measuring cylinder, weighing balance, refrigerator.

2.2 Reagents

Methanol, distilled water, dilute ammonia, hydrochloric acid, ferric chloride, fehling solution, mayer's reagent, Brady's reagent, Dragendroff's reagent, tetraoxosulphate(vi) acid, iodine, ferrous sulphate, ammonium chloride, petroleum ether, acetone. All the reagents are of analytical grade.

2.3 Collection and Preparation of Samples

The leaves of *Vernonia amygdalina* and *Jatropha gossypifolia* were collected from a garden in Auchi, Edo State Nigeria. The leaves were identified and authenticated at Pax Herbal and Research Centre Ewu, Edo state. The leaves were destalked, washed and air dried? for two weeks with constant turning to avert fungal growth. The dried leaves were later milled to obtain the vegetable leaf meal using a manual blender and stored in well labeled air-tight containers for analysis. The extracts were extracted with methanol using soxhlet extractor.

2.4 Preparation of Reagents

2.4.1 10% dilute ammonia solution

- 10 ml of concentrated ammonium was dissolved in 90 ml of distilled water.
- 1% hydrochloric acid
- 1 ml of concentrated hydrochloric acid was dissolved in 90 ml of distilled water.
- 0.1% ferric chloride
- 0.1 g of ferric chloride into 100 ml volumetric flask to fill up to the mark with distilled water.

2.4.2 Fehling solution

- **Solution A:** ($CuSO_4$) 3.5 g of copper sulphate was dissolved in 50 ml of distilled water
- **Solution B:** (NaOH) 3 g of sodium hydroxide was dissolved in 25 ml of distilled water.
- **Solution C:** (NakTatarate) 8.65 g of sodium potassium tartrate was dissolved in 25 ml distilled water.

Solution A, B, C were added to make up 100 m [11].

2.4.3 Mayer's reagent

- **Solution A:** 1 g of mercury chloride was dissolved into 60 ml of distilled water.
- **Solution B:** 5 g of potassium iodide was dissolved into 20 ml of distilled. Then solution A and B were mixed together into 100 ml volumetric flask and add up to the mark [12].

2.4.4 Dragendroff's reagent

- **Solution A:** 0.17 g of bismuth nitrate in 2ml of glacial acetic acid and add into 8 ml of distilled water [13].
- **Solution B:** 4 g of potassium iodide in 10 ml of glacial acetic acid and add 20 ml of distilled water. The two solutions were mixed together in 100 ml volumetric flask and made up to the mark.

2.4.5 Brady's reagent

40 g of 2,4-dinitrophenyldrazine in 80ml concentrated tetraoxosulphate (vi) acid, cool and add 90 ml of methanol and 100 ml of distilled water according to the standard methods of analysis of analytical methods committee of Royal Society of Chemistry, (2002) was adopted.

2.4.6 0.005M of iodine

2 g of potassium iodine into a 100 ml beaker, 1.3 g of iodine and add it into the same beaker and add a few ml of distilled water and swirl for a few minutes until iodine is dissolved. Iodine solution was transferred into a volumetric flask using distilled water and was made up to the mark [14].

2.4.7 Qualitative phytochemical analysis of *Vernonia amygdalina*and *Jatropha gossypifolia*

Phytochemicals screening procedures carried out were adopted from [15]. The analysis determines the bioactive compounds that

contribute to the flavor, colour, and other characteristics of vegetable leaves.

2.5 Phytochemical Screening

2.5.1 Test for alkaloids

3 ml of 1% HCl was added to 3ml of filtrates and then steamed for 30 minutes each. The mixtures were allowed to cool and centrifuged at 3000 rpm for minutes each. Then 3 ml of supernatant of each filtrate was shared in equal proportion into 3 test tubes and labeled A, B, C and 1, 2, 3 respectively. 1 ml portion of the supernatant was treated with 1ml of the following reagents respectively with Dragendroff's reagent, an orange precipitate appears show the presence of alkaloids and with Mayer's reagent a cream white colored precipitate indicated the presence of alkaloid [11].

2.5.2 Test for tannins

2 ml of 0.1% ferric chloride was added to 2 ml of filtrate; blue-black coloration and brownish-green color indicates the presence of tannins [14].

2.5.3 Test for steroid

0.5 ml of extract was added to 0.5 ml of acetic acid anhydride and cooled in ice. 0.5 mml of chloroform and 1ml of concentrated $H_2SO_4$was added carefully with pipette. A reddish-brown ring was formed at the separation levels of the two liquids [14].

2.5.4 Test for flavonoids

5 ml of dilute ammonia and 1ml of concentrated H_2SO_4 was added to 2 ml of the filtrates; yellow coloration reveals the presence of flavonoids which disappears upon standing [14].

2.5.5 Test for polysaccharide/starch

2 ml of filtrate was added to 6 drops of iodine solution. Blue-black coloration reveals the presence of phlobatannin [16].

2.5.6 Test for reducing sugar

2ml of filtrate was added, 5 ml of Fehling solution and steamed for 30 minutes. Red coloration reveals the presence of reducing sugar [17,18].

2.5.7 Test for terpenoids

2ml of extract was added, 6 drops of Brady's reagent. A yellowish-orange colour reveals the presence of terpenoids [11].

2.5.8 Test for cardiac glycoside

2 ml of extract was added, 2 ml of glacial acid, 1 ml of $FeCl_3$, 1 ml of concentrated H_2SO_4 acid. Green-blue coloration indicates the presence of cardiac glycoside [11].

2.5.9 Test for coumarin

2ml of the extract was added few drops of 2M NaOH solution; dark-yellow coloration indicates the presence of coumarin [17,18].

2.6 Quantative Determination of Phytochemicals

2.6.1 Determination of alkaloids

0.5 g of the sample was dissolved in 96% ethanol, 20% H_2SO_4 (1:1), 1 ml of the filtrate was added to 5 ml of 60% H_2SO_4, and allowed to stand for 3 h. The reading was taken at an absorbance of 565 nm [19].

2.6.2 Determination of saponins

0.5 g of the sample was added to 2 ml of 1MHCl and was boiled for 4 h. After cooling, it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 ml of each was taken into 3 different test tubes. 6 ml of ferrous sulfate reagent was added to each followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 minutes and the absorbance was taken at 490 nm [15].

2.6.3 Determination of tannins

5 g of the ground sample was shaken constantly for 1 minute with 3 ml of methanol in a test tube and then poured into a Buchner funnel with the suction already turned on. The tube was quickly rinsed with an additional 3 ml of methanol and the content poured at once into the funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. For aqueous extractions, 5 ml of water was used and for the rinse and the filtrate was added to 50 ml of water. 3 ml of 0.1M $FeCl_3$in 0.1M H_4Cl was added to 5 ml of the extract and followed immediately by timed addition of 3 ml of 0.008 M $K_2Fe(CN)_6$. The absorbance was taken at 720 nm spectro-photometrically [20].

2.7 Fourier Transform Infrared (FT-IR) Analysis

The Fourier Transform Infrared (FT-IR) analysis was carried out in NARICT laboratory, Zaria, Kaduna State, Nigeria using FT-IR Spectrophotometer Model 8400S (Shimadzu Corporaation, Japan).

3. RESULTS AND DISCUSSION

The results of qualitative analysis of *Vernonia amygdalina* and *Jatrophagossypifolia* leaves samples are shown in the Table 1. The results obtained showed the presence of glycoside, saponin, flavonoids, phenolic compounds, alkaloids and reducing sugars.

3.1 Discussion

The results obtained as presented in Table 1 revealed the presence of glycoside, saponin, flavonoids, phenolic compounds, condensed tannin, alkaloids, steroids and reducing sugar. Cardiac glycoside, hydrolysable tannin, phlobatanin, terpenoids, polysaccharide/starch and coumarin were found to be absent. Alkaloids, tannins and flavonoids are some of the most important bioactive components from plants [21]. The occurrence of flavonoids in both *Vernonia amygdalina* and *Jatropha gossypifolia* indicate their anti-bacteria and anti-viral activities [22]. Their activities may be as a result of their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall since they are hydroxylated phenolic substances. They are also effective antioxidant and show strong anticancer activities [23]. Table 2 shows the quantity of some bioactive compounds present in the selected plants such as alkaloids,saponins and tannins. Generally, the samples showed higher levels of these bioactive compounds, the valuable pharmaceutical properties in both samples may be attributed to the presence of bioactive compounds like alkaloids. Alkaloid has been used as central nervous system (CNS) stimulant, topical anesthetic in ophthalmology, powerful pain relievers, antipuretic action, among other uses [24]. Caffeine and theophylline, alkaloids in coffee and tea respectively, prolong or intensify the activity of adrenaline by decreasing the rate of breakdown of cyclic Adenosine Monophosphate (cAMP) [25]. Saponin had been found useful in treatment of hypercholesterolemia, which suggests that the saponins might be acting by interfering with intestinal absorption of cholesterol [26]. The commonly considered anti-nutrient compounds like phenols and tannins are now being considered as potential antioxidants with health promoting effects [27,28,29,30].The mechanism of dietary effect of tannin may be understood by their ability to form complex with protein [31]. Table 3 shows some of the important peaks from infrared (IR) analysis of and *Jatropha gossypifolia* and a commercial anti-malaria drug(Amala). The result shows that they have common peaks and showed similarities in their functional groups, most importantly the presence of Carboxylic acid, Quinone, Aromatic compound, Amine, Alcohol and Aliphatic groups.

Table 1. Qualitative phytochemical data of samples of *Vernonia amygdalina* and *Jatropha gossypifolia*

Parameters	*V. amygdalina*	*J. gossypifolia*
Glycoside	+++	+++
Cardiac glycoside	-	-
Saponins	+++	-
Flavonoids	++	++
Phenolic compounds	+++	+
Hydrolysable tannin(blue-black)	-	-
Condensed tannin(brown-green)	+++	+
Phtobatanin	-	-
Terpenoids	-	-
Polysaccharide/starch	-	-
Alkaloids	++	+++
Steroids	+++	+
Coumarin	-	-
Reducing sugar	+++	+++

Note: + = present; ++ = much present; +++ = very much present;- = Absent

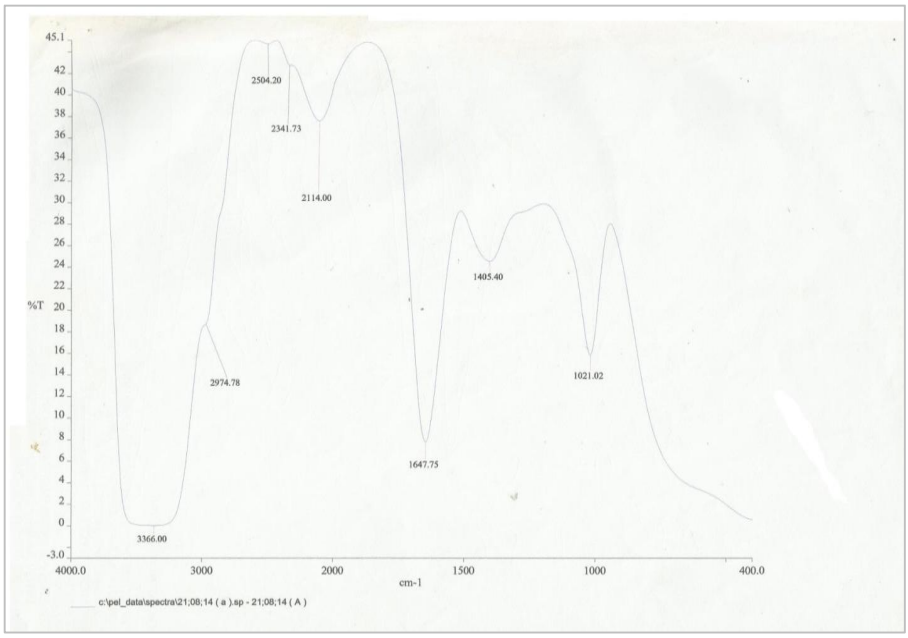

Fig. 1. (Infrared) IR Spectral of *Vernonia amygdalina*

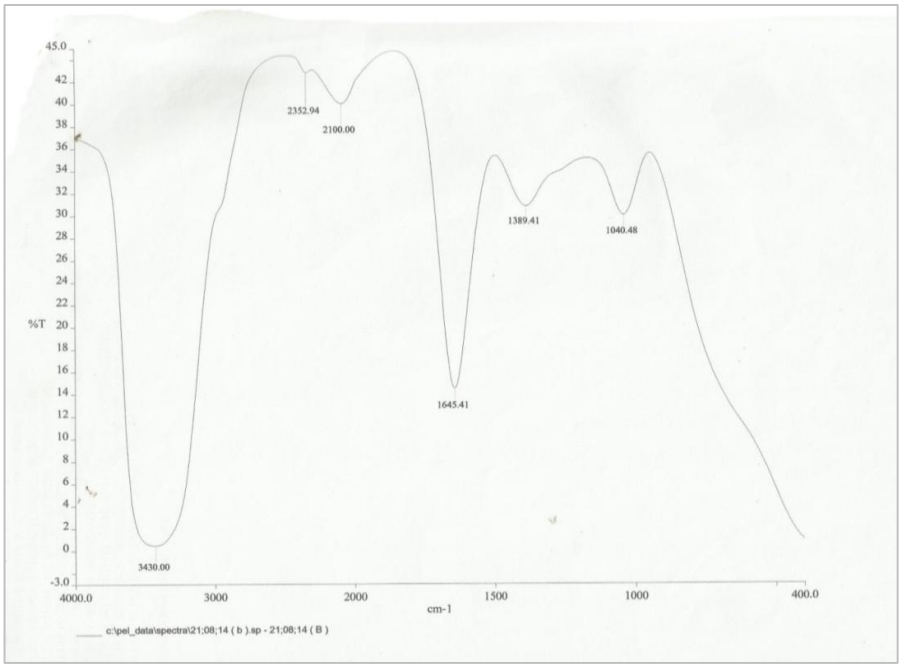

Fig. 2. Infrared (IR) spectral of *Jatropha gossypifolia*

Table 2. Quantitative phytochemical data of *Vernoniaamygdalina* and *Jatrophagossypifolia*

Name of phytochemical	*V. amygdalina*	*J. gossypifolia*
Saponins(g/dm3	1.103 ± 0.019	1.079 ± 0.044
Tannins(g/dm3)	26.48 ± 0.27	24.12 ± 0.17
Alkaloids(g/dm3)	0.580 ± 0.16	0.7585 ± 0.029

Note: data are expressed in Mean±SD from triplicate experiments (n=3) at p<0.05

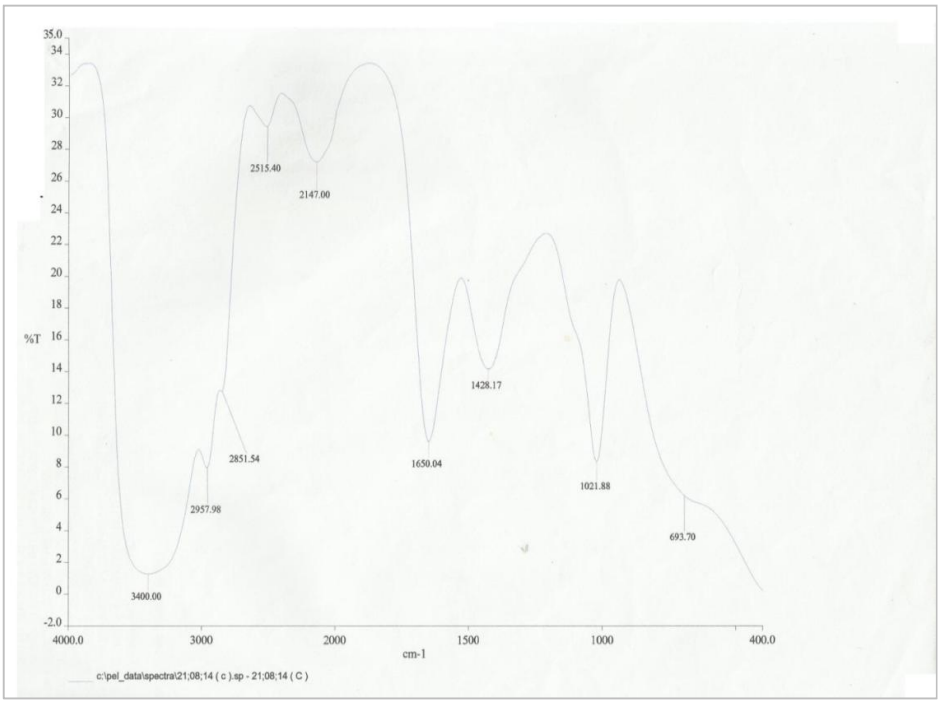

Fig. 3. Infrared (IR) spectral of anti-malaria drug (Amala)

Table 3. IR peak values, probable functional groups and inferences of *V. amygdalina*, *J. gossypifolia* and a commercial anti-malaria drug (Amala)

Samples	Important peak values (CM^{-1})	Functional group	Inference
A: *Vernonia amygdalina*	3366.00	OH Stretch (broad) H bonded	COOH (from carboxylic acid)
	2114.00	C≡C. Stretch terminal alkyne mono-substituted	Alkyne
	164.75	Conjugated Ketone	Quinone
	1405.40	Aromatic carbon-carbon stretch	Aromatic
	1021.02	C-N stretch	Primary amine group
	2974.8	C-H stretch	Aliphatic C-H group
	2504.20	O-H stretch	Alcohol
B: *Jatropha gossypifolia*	3430.00	Hydroxyl group, H-bond (broad) OH stretch	COOH from carboxylic acid
	2100.00	C≡C terminal alkyne mono-substituted	Alkyne
	1645.41	Conjugated ketone	Quinone
	1040.48	C-N stretch	Primary amine
C: Commercial anti-malaria drug (Amala)	3430.00	Hydroxyl group, H-bonded, OH stretch (broad)	COOH from carboxylic
	2957.98	C-H stretch	Aliphatic group
	2851.54	C-H stretch	Methylene group
	2147.00	N≡N stretching	Azide group
	1650.04	Conjugated ketone	Quinone
	1428.17	Aromatic carbon-carbon stretching	Aromatic compound
	1021.88	C-N stretching	Primary amine group
	2515.40	OH stretching	Alcohol

Figs. 1-3 shows the infrared(IR) spectral of *Vernonia amgydalina, Jatropha gossypifolia* and a commercial anti-malaria drug(Amala) and their peak values as stated in Table 3. The functional group of commercial drug (Amala) and plant extract were compared by their peaks in the IR spectra showing similar functional groups. This is in accordance with the work of [32] in a preliminary clinical trial of *V. amygdalina* in which a decoction of 25 g fresh leaves of *V. amygdalina* was 67% effective in creating an adequate clinical response in African patients with mild falciparum malaria of these 32% had complete parasite clearance, unfortunately, 71% had recrudescence. The treatment was without significant adverse effects.

4. CONCLUSION

The results obtained from this work revealed that leaves of *Vernonia amygdalina* and *Jatropha gossypifolia* contain an appreciable amount of flavonoids, saponins, alkaloids and tannins. Considering these substantial amount of bioactive compounds it can therefore be concluded that *Vernonia amygdalina* and *Jatropha gossypifolia* leaves can contribute significantly to the health management of man and should be recommended in our daily nutritional needs.

Based on the results obtained from this analysis, it can be inferred that by further chemical modifications *Vernonia amygdalina* and *Jatropha gossypifolia* could serve as potential anti-malarial drugs which will be cost effective and found within the reach of the poor people since they are naturally and readily available.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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