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Comparison of Yield and Phytoconstituents of *Vernonia amygdalina* and *Ocimum gratissimum* Leaves Extract from Three Extraction Methods

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Abstract

The economical extraction of large amount of specific phytoconstituents with a smaller quantity of solvent requires a simple and safe technique, since extraction is one of the most significant stages in the discovery of new active drug-candidate. This study investigated the phytochemical contents of two medicinal plants, extracted using different extraction methods. Twenty grams of *Vernonia amygdalina* (VA) and *Ocimum gratissimum* (OG) leaves were extracted with three different extraction methods (maceration, Soxhlet and microwave) using methanol. The yield and phytochemical contents were compared. The results showed that the percentage recovery yield of phytochemical content was significantly (p<0.05) higher in the extracts obtained from microwave method (20.55%), when compared to the Soxhlet (18.55%) and maceration technique (15.60%). The extracts obtained from microwave extraction protocol had significantly (p<0.05) higher concentrations in phenolics, saponins and tannins. Soxhlet extraction gave extract with significantly (p<0.05) higher yield on alkaloid, while the extract from maceration was higher in flavonoids. This study showed that the concentration of specific phytoconstituents obtained from medicinal plants depends on the extraction method. The results from this study suggest that Soxhlet technique is best for extraction of alkaloid, maceration technique for flavonoids and microwave assisted extraction technique for phenolics, saponins and tannins.

Keywords

Medicinal Plants, Phytochemical, *Vernonia amygdalina*, *Ocimum gratissimum*, Microwave Extraction, Soxhlet, Maceration

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1. Introduction

The use of plant has been the main source of therapeutic agents from the very beginning of man's origin. Despite the fact that a number of active phyto-constituents have been discovered from plants, yet the quality and safety related problems of herbal drugs (interfering with blood clotting mechanism and increasing the risk of bleeding during surgery, example; Garlic, Ginseng and Ginkgo) has been a challenge for researchers [1]. Bearing in mind also that, the extraction procedure used contributes significantly to the final quality of the herbal drug [2]. Studies have shown that plants whose properties are similar to conventional pharmaceutical drugs in which humans have utilized throughout the history of life

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either to cure or reduce the symptoms resulting from illness are known as medicinal plants [3]. Moreover, such plants have the ability to synthesize varieties of chemical compounds that are useful in important biological functions, and to resist attack on herbivorous mammals, fungi and insects [4].

Treatment of diseases globally with medicinal plants are often more affordable compared to use of orthodox drugs which are expensive modern pharmaceuticals among non-industrialized societies globally [1]. From the estimation of World Health Organization, 80% of the population of some Asian and African countries utilizes herbal medicine for some aspect of primary health care presently [1]. Most of the plants commonly populated in human settlements known to alleviate symptoms of illnesses include: *Azadirachta indica* (Dogonyaro), *Ocimum gratissimum* (Scent leaf), *Allium sativa* (Garlic), *Vernonia amygdalina* (Bitter leaf) and *Zingiber offinale* (Ginger) have medicinal properties [5, 6]. These medicinal properties enhance chemotherapy sensitivity, inhibition of the growth of cancerous cells, possess in-vitro anthelminthic anti–parasitic properties and also serve as antioxidants [7].

Vernonia amygdalina, commonly known as bitter leaf due to its bitter taste is a small shrub with a rough bark that typically grows to a height of 2-5 meter in the tropical Africa [8]. The species of the plant ranges from *V. acaulis, V. arkansana, V. angustifolia, V. baldwinii, V. blodgettii, V. fasciculata, V. faccidifolia, V. gigantea* or *altissima, V. glauca, V. larseniae, V. lettermannii, V. lindheimeri, V. marginata, V. missurica, V. noveborasensis, V. proctorii, V. pulchella* to *V. texana* in North America and *V. nonoensis, V. patens, V. scorpioides, V. condensata* in South America. In addition, *V. bamendae, V. calvoana, V. colorata, V. galamensis, V. staehelinoides* to *V. amygdalina* in Africa and *V. arborea, V. cockburniana, V. elaeagnifolia, V. unicata* to *V. zollingerianoides* in Asia [9]. *Vernonia amygdalina* are found naturally in forest margins, woodland, along rivers, lakes, and grassland, up to 2000 m altitude. It occurs frequently in disturbed localities such as abandoned farmland, and it also grows spontaneously in secondary forest which requires full sunlight in cultivation. The plant prefers a humid environment though it is fairly drought tolerant and can be found on all soil types, but performs best in humus-rich soils and reproduces by hybridizing between similar species in areas of overlapping ranges [10]. It is documented that this valuable medicinal plant can be used commonly in traditional medicine and the leaf decoctions are used for the treatment of malaria [11], fever, headache, scabies, diarrhea, dysentery, hepatitis, stomach-ache, cough, as a laxative and as a fertility inducer [12]. In Nigeria and many West African countries like Cameroon and Ghana, the leaves are placed on wound as a substitute for iodine and as a treatment against intestinal worms including nematodes while the root are used as chewing stick and for the treatment of sexually transmitted diseases [13]. Recent report also showed that the leaf fractions have anti-diabetic activity in fortified diet-fed Streptozotocin-treated rat (FDF rat) model of Type 2 diabetes [14]. The aqueous leaf extract of different concentrations showed a dose-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment [15].

Ocimum gratissimum is an aromatic medicinal plant, a perennial herb of about 1-3 m tall possessing an erected stem and round-quadrangular in shape. It is popularly known as scent leaf and is widely distributed in the tropics of Africa and warm temperature regions [16, 17]. *O. gratissimum* can be used to treat a wide variety of maladies, ranging from bacterial infections, diabetes, pain and liver damage [16-19]. The oil extracts obtained from the plant are also effective in fighting strains of *E. coli*, dysentery and typhoid. It is discovered from research that clove basil is effective in treating various veterinary problems, killing worms in goats and to increase libido in laboratory mice [19]. From research, the oil which is aromatic yet deadly, can be used as mosquito repellant and also as an analgesic, though the plant is not used alone to provide this relief, it shows success when administered in unison with other proven antibacterial and anti-inflammatory herbal agents from Africa [20]. The bioactive constituents in these medicinal plants responsible for their various uses such as anthraquinones, glycosides, cardiac glycosides, saponins, steroids, triterpenes, tannins, flavonoids, alkaloids and phenol compounds are non-nutritive plant chemicals that have protective or disease preventive properties known as phytochemicals [21].

Phytochemicals produce definite physiological actions in the human body. In standard extraction procedures, phytochemicals can be separated in medicinally active portions of plant from the inactive components using selective solvents. Thus, the standardization of extraction procedures contributes significantly to the final quality of herbal drugs [22]. For these reasons, this study aimed to compare three methods used for the extraction of medicinal plants component so as to ascertain the best method for extraction of phytochemicals in terms of yield, time and volume of solvents used both for industrial and personal laboratory research.

2. Materials and Method

2.1. Sample Collection and Identification

The plant samples (*V. amygdalina* and *O. gratissimum*) were collected from a farm in Samaru in Sabon-Gari, Local

Government Area, (located on latitude: 11°9'55.3" longitude: 7°39'5.84"), Zaria, Kaduna State, Nigeria. The leaves were identified at the herbarium of Biological Science Department Ahmadu Bello University Zaria, Nigeria.

2.2. Sample Preparation

The leaves of the plant were picked off from the stalk, thoroughly washed and air-dried at room temperature. The weights of the leaves were constantly taken to ensure that the samples were completely dried. The samples were ground with mortar and pestle into fine powder. Each of the samples was weighed, 20 grams each for maceration, Soxhlet and microwave was extracted with methanol.

2.3. Sample extraction

2.3.1. Maceration

The method described by Azwanida (2015) was employed [23]. Exactly 20g each of the leaf sample of *V. amygdalina* and *O. gratissimum* were weighed and transferred into two separate beakers of 500 ml each, 150 ml of methanol were added into each samples such that; the solvent was above the samples level and left for 48 hours. The following day, they were decanted into clean beakers using filter paper and rinsed with 100ml of methanol for each sample in order to clean out the plant extract. Each filtrate was made more concentrated using rotary evaporator, then transferred into two separate weighed crucibles and dried using water bath. Thereafter the weight of the crucibles were taken again and stored in the desiccator.

2.3.2. Soxhlet (Hot Continuous Extraction)

The method described by Jensen (2007) was employed [24]. Exactly 20 g of each sample of *V. amygdalina* and *O. gratissimum* leaves were weighed into a paper and was wrapped and placed inside two different Soxhlet apparatus. A condenser and a round bottom flask were fitted to the extractors; 150ml of methanol was placed in the extractors and the temperature of the extractor was set to 64.7ºC (which is the boiling point for methanol). The coloring matter of the samples was allowed to continue siphoning until the solvent became colourless. The samples were then removed from the extract to allow the extracting solvent to be recovered. The time taken were observed and recorded. The extracts were poured into an evaporating dish and were concentrated using rotary evaporator. The concentrated crude extracts were transferred into 2 weighed crucibles and allowed to dry. After obtaining the dried samples, the weight of the crucibles were taken again. The crucibles were kept in a desiccator.

2.3.3. Microwave Assisted Extraction

The method described by Azwanida (2015) was employed [23]. Exactly 20g of each samples of *V. amygdalina* and *O. gratissimum* leaves were weighed and transferred into two separate tight glasswares of 500 ml each, 100 ml of distilled water were added into each samples such that; the plant samples were moist and left overnight (24 hrs). The following day, 50 ml of methanol were added to each of the glasswares of *V. amygdalina* and *O. gratissimum* and the glass wares were placed in the microwave, switched on and set at defrost for 3 minutes respectively. Thereafter the glasswares were brought out and allowed to cool for 10 minutes and 25 ml of methanol each was respectively added to *V. amygdalina* and *O. gratissimum* and the glassware was placed back in the microwave and set for 3 minutes and allowed to cool for 30 minutes again. The procedure was repeated for three more times. After cooling for 30 minutes, they were decanted into clean beakers using a sieve material of 0.45 um and washed with 100 ml of methanol for each sample. The extracts were concentrated in the rotary evaporator and transferred into weighed crucibles and dried. Thereafter the weight was taken again after drying and the crucible was placed in the desiccator for further use.

2.4. Qualitative Phytochemical Analysis

2.4.1. Test for Carbohydrates

The presence of the carbohydrate content in the plant extracts were determined following the method described by Evans and Kamaran [25]. Briefly, few drops of Molisch reagent was added to a small portion of the extract in the test tube and concentrated sulphuric acid was added down the side of the tube to form a lower layer, a reddish colored ring at the interphase was observed indicating the presence of carbohydrate.

2.4.2. Test for Glycosides

Five milliliters of dilute sulphuric acid was added to a portion of the extract and boiled in water bath for 10-15 mins. The solution was then cooled and neutralized with 20% KOH. It was then divided into two portions. To the first portion, 5ml of the mixture of Fehling's solution A and B was added and boiled; a brick red precipitate indicated the release of reducing sugar as a result of hydrolysis of Glycoside [26].

2.4.3. Test for Free Anthracene Derivatives

Exactly 5 ml of chloroform was added to a portion of the extract in a dry test tube, the solution was shaken for at least 5 minutes. Thereafter, it was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. The presence of free anthraquinones was observed as indicated by a bright pink color in the aqueous (upper) layer [26].

2.4.4. Test for Triterpenes

Equal volume of acetic acid anhydride was added to a portion

of the extract, and mixed gently. 1 ml of concentrated sulphuric acid was added down the side of the test tube to form as lower layer. The color changes were observed immediately and over a period of one hour. Blue to blue green color in the upper layer and a reddish color indicated the presence of triterpene [26].

2.4.5. Salkowski Test for Sterols

About 2-3 drops of concentrated sulphuric acid was added at the side of the test tube containing a small portion of the extract. Cherry red color indicated the presence of unsaturated sterols [26].

2.4.6. Test for Cardiac Glycoside

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a layer at the bottom, a pale green color in the upper acetic acid layer indicated the presence of cardiac glycosides [26].

2.4.7. Test for Saponin Glycoside

About 10 ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persisted for 10-15mins indicated the presence of saponins [26].

2.4.8. Test for Tannins

Exactly 3-5 drops of ferric chloride solution was added to a portion of the extract. Greenish-black precipitate showed the presence of tannins [26].

2.4.9. Test for Flavonoids

A portion of the extract was dissolved in 1-2 ml of 50% methanol in the heat Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. The red color indicated the presence of flavonoids *[*25].

2.4.10. Test for Alkaloids

Few drops of Dragendoff reagent was added to a portion of the extract. The result was determined by a reddish brown precipitate indicating the presence of alkaloids [25, 26].

2.5. Quantitative Phytochemical Analysis

2.5.1. Preparation of Fat Free Sample

About 2 g of the sample was weighed and defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours.

2.5.2. Determination of Alkaloid

The analysis was performed according to the method of Harborne [26]. The target of the test was the residue (alkaloid) which was dried to a constant weight and the result obtained.

2.5.3. Determination of Flavonoid

Determining flavonoids was performed following the method described by Bohm and Kocipai-abyazan [27]. The filtrate obtained was weighed to a constant weight and the readings were taken and recorded in mg/g.

2.5.4. Determination of Saponin

The method of Obadoni and Ochuko [28] was adapted in the determination of saponins. The evaporated, dried samples which were obtained from the oven were weighed to a constant weight and recorded.

2.5.5. Determination of Tannin

The determination of tannin was performed following the method described by Van-Burden and Robinson [10]. The absorbance of the sample was measured at 120 nm within 10 min.

2.5.6. Determination of Total Phenols

The total phenolic content was determined using the spectrophotometric method. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic components for 15 minutes. About 5 ml of the extract was pipette into a 50 ml flask. Then 10 ml of distilled water was added. About 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol was added also. The sample was made up to mark and left to stand for about 30 minutes for colour development. This was measured at 505 nm.

3. Results

From the three protocols of extraction of *V. amygdalina and O. gratissimum* leaves, it was observed that the microwave assisted extraction technique gave the highest recovery yield (20.55% and 16.90% for *V. amygdalina* and *O. gratissimum* leaves respectively) using the smallest volume of solvent when compared with Soxhlet and maceration techniques. The maceration extraction technique was observed to have recorded the least recovery yield after 48 h using the highest volume of solvent (Table 1).

The results for the qualitative screening showed that both plants *V. amygdalina* and *O. gratissimum* leaves exhibited comparative result for the phytochemicals tested. Positive for carbohydrates, glycosides, cardiac glycosides, saponin, steroids, triterpenes, tannins, flavonoids, and alkaloids and negative for one anthroquinone (Table 2).

Table 1. Volume of solvent used, duration and yield of extract obtained from *Vernonia amygdalina* and *Ocimum gratissimum*leaves using three extraction methods.

	Vernonia amygdalina			*Ocimum gratissimum*		
	MAE	**Soxhlet**	**Maceration**	**MAE**	**Soxhlet**	**Maceration**
Volume of Solvent (ml)	100	150	250	100	150	250
Extraction Duration (Min)	45	265	2880	45	265	2880
Recovery Yield (g)	4.11 ± 0.69	3.70 ± 0.17	3.12 ± 0.13	3.38 ± 0.89	2.03 ± 0.15	1.85 ± 0.10
Percentage Recovered (%)	20.55	18.50	15.60	16.90	10.15	9.25

Values are mean ± SD of triplicate determinations; MAE: Microwave Assisted-Extraction

Table 2. Phytochemicals present in methanol extracts of *Vernonia amygdalina* and *Ocimum gratissimum* under different extraction methods.

Constituents	*Vernonia amygdalina*			*Ocimum gratissimum*		
	MAE	**Soxhlet**	**Maceration**	**MAE**	**Soxhlet**	**Maceration**
Carbohydrates	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+

Key: (+): Present; (-): Absent; MAE: Microwave Assisted-Extraction

Table 3. Quantitative analysis of phytochemicals of *Vernonia amygdalina* and *Ocimum gratissimum* under different extraction methods.

	***Vernonia amygdalina* (mg/100 g)**			***Ocimum gratissimum* (mg/100 g)**		
	MAE	**Soxhlet**	**Maceration**	**MAE**	**Soxhlet**	**Maceration**
Alkaloids	1.60 ± 0.51^{a}	2.75 ± 0.37^{b}	1.90 ± 0.60^{a}	2.40 ± 0.25^{b}	2.65 ± 0.25^{b}	1.75 ± 0.35^{a}
Flavonoids	5.20 ± 0.15^{b}	3.70 ± 0.25^{a}	6.20 ± 0.15^{c}	3.80 ± 0.16^{b}	3.00 ± 0.20^{a}	4.70 ± 0.24^{c}
Phenolics	6.50 ± 0.38^{c}	5.80 ± 0.40^{b}	4.40 ± 0.50^{a}	6.10 ± 0.30^{c}	5.20 ± 0.43^{b}	4.20 ± 0.30^{a}
Saponins	1.70 ± 0.25^{b}	1.30 ± 0.50^{a}	0.90 ± 0.32^{a}	2.20 ± 0.33^{b}	1.60 ± 0.50^{a}	1.80 ± 0.40^{a}
Tannins	5.70 ± 0.35^{b}	5.30 ± 0.30^{b}	4.00 ± 0.40^{a}	4.85 ± 0.21^{b}	3.62 ± 0.30^{a}	3.10 ± 0.51^{a}

Values are mean ± standard deviation of triplicate determinations. Same superscript across the row under the same plant signifies no significant difference at $p>0.05$. MAE: Microwave Assisted-Extraction

The quantitative analysis of the phytochemical content of the extracts of the two plants derived from the three extraction methods are presented in Table 3. It was observed that extracts obtained through maceration technique had a significant ($p<0.05$) higher amount of flavonoids. The extracts obtained from Soxhlet extraction procedure had the highest amount of alkaloids, whereas, the microwave assisted extraction technique recorded significantly ($p<0.05$) higher amount in phenolics, saponins and tannins in the extracts of both plants (Table 3).

4. Discussion

The comparative evaluation of the extraction method for both plants extracts under investigation in terms of yield, duration and the volume of solvent used, maceration had the least yield, whereas, microwave recorded the least volume of solvent as well as duration of extraction. The explanation to the observed differences in yield of phytoconstituents recovered from the three extraction methods could be due to the heat transfer as in the case of Soxhlet extraction and the mass transfer which are limiting factors compared to microwave heating, where heat transfer takes place from the core of the samples to the external colder environment. Additionally, the volumetric heating effect leads to a rapid increase in temperature. Furthermore, the interior heating of *in situ* water within the plant matrix expands the plant cells and results to the breaking of the glands [29]. This could be the rationale in the significant ($p<0.05$) higher yields of phytochemicals obtained from MAE method when compared to the Soxhlet and maceration extraction methods in the present study.

The secondary metabolites (such as alkaloids, phenolics, flavonoids, saponins and tannins) which are the bioactive principles responsible for medicinal values of the respective plants were all present. These phytoconstituents were detected in varied concentrations in the extracts obtained from the different extraction protocols from the two plants using methanol. This observation was synonymous with recent reportsin terms of the duration observed for the microwave assisted extraction [30]. On the other hand, the observed differences in Soxhlet and maceration yield between the present report and that of Okoduwa *et al* [30] could be due to

differences in the location and time of harvesting of the plants. Location and time of plants harvesting are known to play significant role in the quantity of phytochemical composition present in a plant [31, 32]. In the present study, the same specific phytochemicals (phenolics, saponins and tannins) which were extracted in significant higher amount using MAE method from both plants: *V. amygdalina* and *O. gratissimum* leaves (Table 3), suggests that MAE is a better method for the extraction of these phytochemicals from *V. amygdalina* and *O. gratissimum* leaves. Although, Soxhlet extraction technique gave higher yield in alkaloids and the maceration extraction technique was higher for the extraction of flavonoids, the MAE was more economical as it required shorter operational time with the least volume of solvent (Table 1). In a previous study, Proestos and Komaitis, [33] reported that MAE possess moderately high recoveries with good reproducibility and minimal sample manipulation for extraction process [33]. Numerous bioactive components have been extracted using MAE. For instance, extraction of artemisinin from *Artemisia annua* [34], Quercetin from herbal plant [35] and extraction of Azadiractine related limonoids from *Azadirachta indica* seed kernels [36]. The extraction of phenolic substances from the peel of *Dimocarpus Longan* using MAE was significantly higher than that obtained using Soxhlet extraction [37].

The phytochemical analysis revealed the presence of tannins, alkaloids, saponins, flavonoids, phenolics and carbohydrates. This was similar to the report of Ramamoorthy *et al*. [38], on a preliminary phytochemical analysis and quantification of total phenols and antibacterial activities of methanol extracts of *V. amygdalina* and that of other investigators that did related work in screening the methanol extract of the leaves of *O. gratissimum*, an important and endemic medicinal plant of Kashir Himalaya for the presence of various bioactive plant metabolites and analgesic activity [30, 38, 39]. The findings of this study also agrees with the report of Kumudhavalli and Jaykar [40] on the evaluation of petroleum ether, chloroform, acetone, ethanol and aqueous extract of the plant *V. amygdalina* for preliminary phytochemical screening. Maraleedharannair et al. [41] have reported the presence of flavonoids, carbohydrates, phenolic compounds and sterols as the major phytoconstituents in both ethanol and aqueous extracts of *V. amygdalina*. The extracts of *V. amygdalina*, *A. caudatau* and *C. parasitica* showed varied degree of phytoconstituents with reference to solvent in a similar manner to our present study, but there was a slight difference in our result as compared with that of Kunda and Rajukar [42] on the screening of *V. amygdalina* for phytochemical constituents.

5. Conclusion

This study demonstrated that microwave assisted extraction technique represents a promising substitute for extracting specific phytoconstituents from natural substrate. The results of our study on the methods of extraction of medicinal plants in terms of amount of solvent used, yield and duration suggest that microwave assisted extraction protocol is the best method suited for highest yield. The findings obtained from the present research showed that the choice of extraction techniques should be based primarily on the phytochemical entity of interest. The results obtained from this study reveals that maceration extraction technique is best when the phytochemical of interest is flavonoids, Soxhlet technique if alkaloids is needed and microwave assisted extraction protocol if phenolics, saponins and tannins are the phytochemical of interests. Further study is recommended to evaluate the biological efficacy of the phytochemicals obtained via different extraction methods.

Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration between all the authors. Author "S.I.R.O" got the concept, design of the study, its interpretation and critically revised the draft manuscript for important intellectual content. Authors "L.O.M", "B.E.I" "U.J.O" participated in the acquisition of data, laboratory aspect of the work and drafted the manuscript. Authors D.H.M" and "O.O" managed the literature search and participated in data validation, statistical analysis and subsequent revision of the final version. All the authors read through the manuscript and gave a final approval of the revised version to be published.

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