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Effect of Extraction Solvent on the Phenolic Content, Flavonoid Content and Antioxidant Capacity Of Clove Bud

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Abstract: *In this research, the effect of extraction solvent on the phenolic content, Flavonoid content and antioxidant capacity of clove bud (Syzygium aromaticum) was investigated. The extraction of clove bud was carried out with water, methanol (80%) and acetone (80%). The Flavonoid and phenolic contents of the extracts were evaluated by standard Aluminum chloride and Folin-Ciocalteu methods respectively while their antioxidant capacity was assayed by determining their total reducing power as well as their free radical scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical. The percentage yield of the crude extracts ranged from 15.1±0.32% to 18.33±0.41% with the 80% acetone showing the highest yield while water extract showed the least. The flavonoid contents of all the extracts of clove bud evaluated were higher than the phenolic contents. The highest flavonoid content (501±0.58mgQE/g), phenolic content (200.2mgGAE/g), average percentage DPPH free radical inhibition (73.67±0.34%) and highest reducing power (0.88±0.10) were exhibited by the 80% acetone extract. The water extract on the other-hand gave the lowest values for all the parameters tested with 161.67±0.88mgQE/g, 118.40±0.06 mgGAE/g, 62.05±0.22% and 0.76±0.01 for Flavonoid content, phenolic content, average DPPH free radical inhibition and reducing power respectively. A positive correlation was found between the concentrations of flavonoids and phenolics and the antioxidant capacity of the different solvent extracts of clove bud. From the results of this study, it was shown that the flavonoid and phenolic contents of clove bud are best extracted with aqueous semi-polar organic solvents such as aqueous acetone since the most polar solvent; water, gave the least concentration of the phytochemicals determined in this work. This study therefore showed that extraction solvent is not only a crucial determinant in phytochemical recovery from plants but also an important factor to note when considering the antioxidant capacity of plants extracts. Further work is recommended to determine a detailed chemical composition of the different solvent extracts of clove bud in other to ascertain the presence of other phytochemicals which in addition to the phenolics and flavonoids may account for the antioxidant activity of these extracts.*

Keywords*: Antioxidants, Flavonoids, Percentage yield, Phenolics, Syzygium aromaticum*

I. Introduction

The importance of natural products in both traditional and modern time medicines cannot be over-emphasized due to their immense contributions to the well-being of man. This may be attributed to their possession of rich phytochemicals which are known to have both preventive and curative/therapeutic properties against wide spectrum of diseases. In recent times, the use of naturally sourced antioxidants and other pharmaceutics has gained considerable attention from researchers due to their presumed safety, nutritional and high therapeutic values. Extraction solvent and the method of extraction are however some of the many factors which are pivotal to the recovery of these medicinal phytochemicals from natural products. Some of these extraction solvents which include water, alcohols, acetone or their mixtures are commonly used to extract phytochemicals from plants, and they influence both extraction yield and antioxidant activity of extracts. It has been reported that the complexities of both the chemical properties of extraction solvents and the varied structure and composition of the plant materials make the behavior of material-solvent systems different from each other and also difficult to predict [1]. Hence, no single solvent is capable of extracting all the antioxidants of different polarity and solubility in a single plant. It is therefore essential not only to determine that natural products such as green plants or spices possess therapeutic components but to also find out which solvent or solvent mixture is most suitable for the extraction of these phytochemicals from the plants.

Clove (*Syzygium aromaticum*) is one of the most commonly used spices around the world including Nigeria in Africa as preservative, medicine as well as in the preparation of various spicy rich dishes. The clove tree is a perennial tropical plant which grows to a height of about 10-20meters, having large oval leaves and crimson flowers in numerous groups of terminal clusters [2]. Clove bud is the dried, unopened, nail-shaped, flower bud of the evergreen *Syzygium aromaticum* tree. Clove bud is aromatic, a stimulant and carminative and is used for dyspepsia and gastric irritation [3]. In addition to its flavoring, pungent and fragrant properties as well as culinary uses, the clove bud and its oil have an abundance of medicinal and recreational uses. The use of clove bud in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment and condiment with carminative and stimulant activity has been reported [4]. It is also used in dentistry as a natural analgaesic

and antiseptic due to its possession of eugenol; its major component [5]. Clove oil; an essential oil derived from the dried flower buds, leaves and stems of *Syzygium aromaticum* tree has a spicy aroma which makes it a sought after ingredient for food flavoring, fragrance formulations as well as in the production of soaps and detergents. The insecticidal properties of clove oil have been studied against lice, weevils, moth caterpillars, beetles and cockroaches [6]. It has also been found to possess mosquito-repelling properties [7, 8]. Clove oil contains several compounds with eugenol been the major component. Other compounds that have been determined to be present in clove oil include β-caryophyllene, eugenyl acetate, α-humulene and others. The oil of clove has also been reported to be a good remedy for bronchitis, the common cold cough, fever and sore throat [5].

Clove buds extracts and especially its oil have been reported to possess anti-oxidant, anti-fungi, anti-viral, anti-microbial, anti-diabetic, anti-inflammatory, anti-thrombotic, anesthetic, pain-relieving and insect repellent properties [9, 10]. Some Polyphenols such as Gallic acid, Ellagic acid, Quercetin glycoside, Ellagic acid derivative and some unidentified phenolic compounds have been characterized and reported to be present in clove buds [11].

This research therefore aimed to evaluate the effect of extraction solvent on the phytochemical constituents' recovery and antioxidant capacity of clove bud extracts in order to determine the solvent system that will best extract polyphenols such as flavovoids and phenolics from it and in relation to their antioxidant activity.

II. Materials And Methods

2.1 Sample collection and preparation

The clove bud used for this research was purchased from a commercial seller in a local market in Ibadan, Oyo state, Nigeria. The clove bud was air-dried and pulverized with the use of a blender (LEXUS MG-2053 OPTIMA). 50 grams of the pulverized sample was macerated with 250ml of different solvents viz; water, methanol (80%) and acetone (80%) for 72hrs. Each was filtered using Whatman No.1 filter paper and the residue was re-macerated for another 24hrs (twice) with equal volume of solvent to ensure adequate extraction. The filtrates of each extract were combined and concentrated using rotary evaporator (Eyela N-1001) at 40 ^{o}C. The resulting crude extracts were weighed and stored at 4^{o}C for further analysis.

2.2 Total flavonoid content

The total flavonoid content of the extracts was estimated spectrophotometrically by the Aluminum trichloride method described by [12]. One millilitre of each extract (containing 1mg/ml) was diluted with distilled water (4ml) in a 10ml volumetric flask. 5% $NaNO_2$ solution (0.3ml) was then added to each volumetric flask. At 5mins, 10% $AlCl_3$ (0.3ml) was added and at 6mins, 1.0M NaOH (2ml) was added. Distilled water (2.4ml) was then added to the reaction flask and shaken thoroughly. Absorbance of the reaction mixture was then read at 510nm.The test was carried out in triplicates. A calibration curve was prepared using a standard solutions of Quercetin (2 to 10μg/ml, R^2 = 0.986) and treated similarly as the sample. Total Flavonoid Content was estimated from Quercetin calibration curve (R^2=0.9972) and results expressed as mg Quercetin Equivalent per gram (mgQE g-1) of the sample on a dry weight basis.

2.3 Total phenolic content

The total phenolic content of the extracts was assayed by the method described by [13]. This estimation is based on the reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds contained in the extracts. The reduced Folin-Ciocalteu reagent was blue and thus detectable with a spectrophotometer at 760nm.

One milliliter of extracts (containing 1mg/ml) in methanol was diluted with water (9ml) in a volumetric flask. Folin-Ciocalteu's reagent (1ml) was added to the mixture and vortexed. After 5 min, 10 ml of sodium carbonate solution (7%) was added to the mixture, and then incubated for 90mins at room temperature. After incubation, the absorbance against the reagent blank was determined at 760nm. A reagent blank was prepared using distilled water instead of the plant extract. The concentration of phenolic compound in the extract was determined from the standard curve prepared from varying concentrations (2-10 μg/ml, R^2=0.998) of Gallic acid analyzed in the same manner as the extract samples and results expressed as mg Gallic Acid Equivalent per gram (mgGAE g-1) of the extract. All samples were analyzed in triplicates.

2.4 DPPH free radical scavenging activity

The antioxidant activities of the extracts were evaluated spectrophotometrically through free radical scavenging effect of the extracts on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method described by [14].

One milliliter of methanolic DPPH solution (0.3mM) was added to 2.5ml solution of the extract of various concentrations(10, 20, 40, 60, 80 and100µg/ml) prepared in methanol or Gallic acid standard (2, 4, 6, 8 and 10µg/ml). The resulting mixture was kept at room temperature for 30mins and the absorbance was read at 518nm. The absorbance was converted to percentage inhibition using the expression;

$$\% \text{ inhibition of DPPH} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

A mixture of 2.5ml Methanol and 1 ml of 0.3mM DPPH was used as the control. The concentration of the extract which caused 50% inhibition of radical formation was represented as IC50 and was obtained by interpolation from linear regression analysis [15].

2.5 Total Reducing Power

The total reducing power of the extracts was assayed according to the method described by [16].
Ome milliliter of various concentrations of extracts (10 µg/ml, 50 µg/ml & 100 µg/ml) were mixed with phosphate buffer (500 µl, 20 mM, pH 6.6) and 1% Potassium Ferricyanide (500 µl). The mixture was incubated at 50^0C for 20 min; after which 500 µl of 10% Trichloroacetic acid was added, and the mixture was centrifuged at 2500 rpm for about 10 min. After centrifuging, the supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300µl) and the absorbance was read at 700 nm. Gallic acid solutions of various concentrations (2, 4, 6, 8 and 10µg/ml) were similarly analyzed. The analysis was done in triplicate. Increase in the absorbance of the reactions mixture indicated increase in the reducing power of the extracts. The IC50 representing the extract concentration which shows absorbance value of 0.5 was calculated from the graph of absorbance at 700 nm against extract concentration [17].

III. Results And Discussion

3.1 Percentage Yield

The percentage yields of clove bud extracts in different solvents are represented in Table 1. From the results, 80% acetone gave the highest yield of clove bud extract (18.33%) followed by the 80% methanol (16.05%) while the lowest yield of extract (15.1%) was obtained for water. This may imply that clove contains more semi-polar constituents than polar ones. This however does not necessarily imply that its polar constituents are less biologically active than the semi-polar or even the non-polar ones. Rather, the highest extraction yield exhibited by the 80% acetone may indicate superior efficacy of this solvent system to solubilize and recover greater amount of extractable antioxidant constituents from clove bud. Variations in the polarity of the extraction solvents, hence different extractability of the antioxidant constituents may also be responsible for the differences in the yield of extracts in different solvents.

3.2 Total Flavonoid and Phenolic Contents

Table 2 shows the results of the total flavonoid and phenolic contents of the various extracts of *S. aromaticum* bud. The highest flavonoid and phenolic contents were obtained for 80% acetone followed by the 80% methanol and the least were obtained for the water extract. This implies that the bioactive compounds or antioxidant phytochemicals (flavonoids and phenols) in clove bud are best extracted with of semi-polar solvents such as aqueous acetone. It is important to note however that the Flavonoid content of each extract was higher than its phenolic content. This may imply that clove bud has more flavonoids than phenols or simply that the extraction solvents were able to extract more flavonoids than phenols from clove bud. There is also a correlation between the extract yield and the concentrations of these polyphenols since 80% acetone with the highest extract yield also gave the highest concentrations of the polyphenols while the least polyphenols' concentrations were obtained for water extract which showed the lowest extract yield. Therefore, of the three extraction solvents used, aqueous acetone (80%) was found to be the most effective and suitable solvent for the recovery of higher amount of phenolics and flavonoids from the bud of clove. The variation in the recovery of flavonoids and phenolics from natural products may be influenced by the type of plant material, the chemical nature of the extractable compounds and the effectiveness of extraction solvents to solubilize such compounds [18, 19]. Solvents with intermediate polarity are reportedly preferred to be used in extraction of phenolics and antioxidants as compared to those highly polar such as water or non-polar solvents such as hexane [20].

3.3 Antioxidant Capacity

Antioxidant capacity of the clove bud extracts was estimated by determining their DPPH scavenging ability as well as their total reducing power. The results of the DPPH scavenging activity and total reducing power of the extracts as given in Table 3 showed that the best DPPH free radical scavenging activity and the

total reducing power were exhibited by 80% acetone extraction followed by 80% methanol and the least was exhibited by the water extract. This implies that the polar constituents of clove bud have lesser antioxidative strength than the semi-polar ones. This clearly showed that extraction solvent is not only a crucial determinant in the level of phytochemical recovery but also an important factor to note when considering the antioxidant capacity of plants extracts. The antioxidant capacity as well as the yields of plant extracts has been reportedly dependent on the nature of selected extraction solvent [21] and this could be due to the presence of different antioxidant compounds of different chemical characteristics and polarities that may or may not be soluble in a particular solvent [22]. Different extractability of antioxidant constituents by extraction solvents due to variations in the polarity of the solvents may be responsible for the differences in the antioxidant activity of extracts in different solvents. It has also reported that aqueous organic solvent extracts of some tested plant materials exhibiting greater total phenolic content and also exhibited good reducing power [22]. This implies that reducing power of plant extracts is associated with their phenolic contents i.e. plant extracts with higher levels of total phenolics exhibit greater reducing power as reported by [23].

In this present investigation, a direct positive relationship existed between the Flavonoid content, phenolic content and the antioxidant activity of the clove bud extracts. This is because highest antioxidant capacity was exhibited by 80% acetone extract which also showed highest concentrations of flavonoids and phenolics. Likewise, water extract with the lowest concentrations of flavonoids and phenolics exhibited the least antioxidant activity.

Phenols and polyphenolic compounds, such as flavonoids, are widely present in food materials derived from plant sources, and they have been shown to possess significant antioxidant activities [24]. Correlation between total phenolic contents and antioxidant capacity of various plants such as fruit and vegetables has been reported [25, 26, 27, 28]. Several authors [29, 30, 31] have also reported the significant contribution of phenolic compounds in spices and herbs to their antioxidant activities. Other researchers [32, 33] have also attributed the antioxidative effects of many plants to the presence of flavonoids, phenolic acids, and phenolic diterpenes. The presence of these phytochemicals; flavonoids and phenolics in clove bud extracts in the order; 80% acetone > 80% methanol > water extract may therefore be largely responsible for the extracts' antioxidant capacity which was in the order 80% acetone > 80% methanol > water. It is important to note however that there are also studies which have reported no positive correlation between the amount of phenolics and antioxidant activity of plants [34, 35] but our work did show a positive correlation. Shan reported a positive correlation of polyphenolic constituents of clove buds and its antioxidant activity [29]. However, even though flavonoids and phenolics have been known to possess antioxidant activity, the antioxidant capacity of plants is not restricted or limited to the presence of these two phytochemicals alone. In other words, antioxidant activities of plant extracts could also be due to the presence of other secondary metabolites such as volatile oils, carotenoids, vitamins and so on [36] or even to synergistic action of various classes of phytochemicals present in the plant.

IV. Conclusion

The results of this study showed that the three extracts of clove bud (water, 80% methanol and 80% acetone) showed very good antioxidant capacity. However, based on the findings of this research, it could be concluded that extraction solvent played a crucial role not only in the extraction of phytochemical constituents of clove bud but also in determining the antioxidant capacity of the extracts. Water which is the most polar solvent gave the lowest extract yield and its extract also showed the least concentrations of both phenolics and flavonoids while the aqueous acetone with the least polarity gave the highest extract yield and its extract showed the highest concentrations of both phenolics and flavonoids. Worthy of note is the fact that the aqueous acetone extract which showed the highest concentrations of phenolics and flavonoids determined also showed the highest antioxidant capacity while the water extract with the least concentration of these phytochemicals showed the least antioxidant capacity. This showed a positive correlation between the phenolic and Flavonoid contents of clove bud and its antioxidant capacity. Meanwhile, the antioxidant activity of clove bud may be due the presence of other undetermined phytochemicals or the synergistic effect of all the phytochemicals that may be present. It is therefore recommended that further and more comprehensive work be carried out on these clove bud extracts in order to determine a detailed chemical composition of the different solvent extracts of clove bud. This may also give better information on other pharmacological usability of each of these extracts.

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Table 1: Percentage yield of *S. aromaticum* in different solvents.

Extract of *S. aromaticum*	PERCENTAGE YIELD (%)
WATER	15.1±0.32
METHANOL (80%)	16.05±0.17
ACETONE (80%)	18.33±0.41

Data are expressed as mean ± standard error of three replicates.

Table 2: Total flavonoid and phenolic contents of water, methanol (80%) and acetone (80%) extracts of *S. aromaticum*

Extract of *S. aromaticum*	Flavonoid content mgQE/g.	Phenolic content mgGAE/g.
Water	161.67±0.88	118.40±0.06
Methanol (80%)	318.67±0.88	170.90±0.06
Acetone (80%)	501±0.58	200.20±0.09

Data are expressed as mean ± standard error of three replicates.

Table 3: Antioxidant activities of water, methanol (80%) and acetone (80%) extracts of *S. aromaticum*

Extract of *S. aromaticum*	DPPH SCAVENGING ACTIVITY		REDUCING POWER	
S. aromaticum	Average % Inhibition	IC50 (µg/ml)	Average Absorbance	IC50 (µg/ml)
Water	62.05±0.22	39.07±0.14	0.76±0.01	16.96±1.30
Methanol (80%)	71.09±0.13	14.58±0.21	0.81±0.03	7.24±0.30
Acetone (80%)	73.67±0.34	7.82±0.06	0.88±0.10	1.68±0.04

Data are expressed as mean ± standard error of three replicates