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ORIGINAL ARTICLE

Quantitative Phytochemical Constituents and Antioxidant Activities of the Mistletoe, *Phragmanthera capitata* (Sprengel) Balle Extracted with Different Solvents

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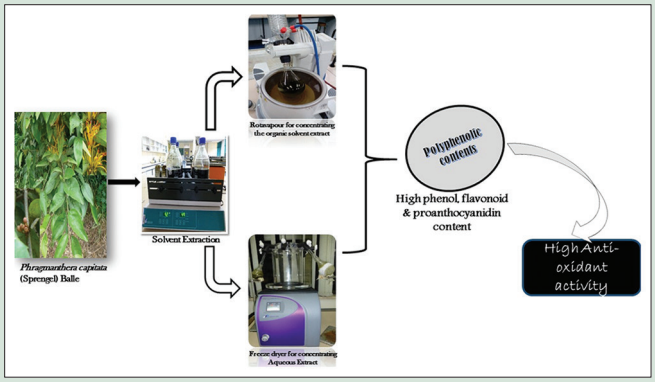
ABSTRACT

Objective: The investigation aimed to evaluate the *in vitro* quantitative phytochemical compositions and antioxidant capacity of the dried leaves of *Phragmanthera capitata* extracted with different solvents. **Methodology:** Different phytochemical assays were used to evaluate the phenols, flavonoids, and proanthocyanidin contents of the acetone, methanol, ethanol, and aqueous extracts of the sample. Antioxidant activity was measured using 2,2diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Ferric reducing antioxidant power (FRAP), nitric oxide, and total antioxidant capacity (TAC) assays. **Results:** Methanol had the highest extraction yield (21.50%) followed by aqueous (10.87%). Acetone and ethanol yielded 6.87% and 4.3%, respectively. However, acetone and ethanol extracts had more phenolic content (218.6 ± 3.38 and 214.8 ± 3.56 mg gallic acid equivalent (GAE)/g, respectively) that were significantly higher ($P < 0.05$) than the methanol and aqueous extracts (203.8 ± 1.95 and 175.5 ± 0.32 mg GAE/mg, respectively). Acetone extract also had more flavonoid and proanthocyanidin contents (679.8 ± 6.26 mg quercetin equivalent/g and 645.7 ± 6.35 mg catechin equivalent/g, respectively) that were significantly higher than the other solvent extracts ($P < 0.05$). All the solvent fractions showed great antioxidant activities with the acetone fraction having the highest capacity based on ABTS, DPPH, and TAC assays (half maximal inhibitory concentration [IC_{50}] = <5 μg/mL, 24.5 μg/mL, and 85 μg/mL respectively). The methanol extract however had FRAP and nitric oxide antioxidant activities (IC_{50} = 302 μg/mL and <25 μg/mL respectively). **Conclusion:** The findings of this study provide evidence that *P. capitata* has potential as a natural source of antioxidant with acetone showing to be the best extractant of the polyphenolics with corresponding higher antioxidant activities.

Key words: Extractant, *Loranthaceae*, mistletoe, *Phragmanthera capitata*, polyphenolics, total antioxidant activity

SUMMARY

- The study investigated the *in vitro* polyphenolic content and antioxidant activities of the mistletoe; *Phragmanthera capitata,* extracted with different solvents
- The resultant outcome of the study showed that while methanol had more yield from the extraction, the highest polyphenolic contents was observed in the acetone extract
- The acetone extract also showed the highest antioxidant activity in most of the assays
- There was a positive correlation between the polyphenolics and the antioxidant activities: Higher polyphenol content resulted in a better antioxidant activity
- If extracting this plant for preparatory works only, methanol would be preferred, but if it were for bioassay screening, acetone will be the solvent of choice.



Abbreviations Used: DPPH: 2, 2diphenyl-1-picrylhydrazyl (DPPH) ABTS: 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) FRAP: Ferric reducing antioxidant power TAC: Total antioxidant capacity GAE: Gallic acid equivalent QE: Quercetin equivalent CE: Catechin equivalent BHT: Butylated hydroxyl toluene.

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INTRODUCTION

Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. These phytochemicals are known to combat diseases due to their antioxidant effect.[1] As a way of responding to environmental stress, many plants produce antioxidant compounds such as the polyphenolics. On the other hand, antioxidants are phytochemicals that mop up and neutralize free radicals, quenching singlet, and triplet oxygen or inducing expression of peroxides and other toxic metabolites. Hence, antioxidants have protective ability from damages resulting from stress caused by free radicals as a result of oxidation.[2]

A large portion of the world's population still relies on alternative medicines for the management and treatment of a large number of

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serious ailments.[3] Medicinal plants represent a lot of untapped natural reservoirs of potential drugs, and the diversity of their structural components makes a valuable source of novel compounds. Hence, there is an increasing interest by natural product scientists in the utilization of phytochemicals and a simultaneous intensifying effort towards the evaluation of these valuable medicinal plants.

One of the various families of medicinal plants that have been exploited in various herbal folklores is the *Loranthaceae*. It is one of the five families in the group of plants called "mistletoe". Species of the *Loranthaceae* have been extensively used in alternative medicine for the treatment and management of several ailments/diseases.[4] Chemical and pharmacological studies of this family have identified several compounds such as flavonoids, alkaloids, lectins, polypeptides, arginine, glycosides, gallic acid, and Loranthin (flavocoumarin).[4] *Phragmanthera capitata* (Sprengel) Balle which is a mistletoe in the *Loranthaceae* family has been used in herbal medicine in Africa. It is the major *Loranthaceae* species used by the traditional healers in the Logbessou region in Cameroon. It has been used for the management and treatment of several ailments which include; convulsions, nerves attacks, chronic muscular pains, diabetes, rheumatism related pains, respiratory dysfunctions, dizziness, epilepsy, uterine hemorrhage, hypertension, hypotension, back pains, kidney pains, menopause, headache, general purifications, heart palpitations, irregular menstruations, and nose bleeding.[5]

In Africa, much attention in mistletoe research is in crop protection, and little has been given to its potential roles in ethnopharmacology (as with the issue of *P. capitata* and rubbers trees). However, there is the need for scientific screening of the African mistletoe for its potential in therapeutics. The chemical profile/constituents of mistletoe chiefly depend on the host plant.[6] Different solvents have been used to extract and isolate bioconstituents in plants, and the group of compounds extracted or isolated is highly solvent dependent.[2] Before this study, no report on the quantitative phytochemical evaluation of *P. capitata* parasitic on rubber trees have been investigated. Hence, the aim of this study was to examine the quantitative phytochemical constituents and their corresponding antioxidant activities of *P. capitata* extracted with different solvents.

METHODOLOGY

Location and collection of sample

The leaves of the mistletoe were harvested from mature rubber plantations in the Rubber Research Institute of Nigeria. The site is located on latitude 6°00'-6°15' N; longitude 5°30'-5°45' E and at about 27 m above sea level. The sample was authenticated by Dr. Emmanuel I. Aigbokhan, of the Plant Biology and Biotechnology Department, University of Benin, where a voucher specimen (UBH10284) was deposited at the University herbarium for future reference.

Extraction procedure

The leaves were separated from the twigs, gently rinsed to remove dust, and dirt and air dried at room temperature (mean morning and night temperature of 24°C and mean noon temperature of 27°C) in a well-aerated atmosphere and prevented from direct sunlight to avoid denaturation of vital phytoconstituents. Dried leaves were pulverised. 300 g each of the ground sample was soaked in separate conical flasks with 1.2 L of the solvents; acetone, methanol, ethanol, and water and shaken in an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 24 h. Crude extracts were filtered with a Buchner funnel and Whatman No. 1 filter paper. While the acetone, methanol, and ethanol extracts were concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator (Strike 202 Steroglass, Italy), the aqueous filtrate was concentrated to dryness using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY).

Reagents and chemicals used

Solvents and chemicals used include; Folin-Ciocalteu, anhydrous sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum trichloride ($AlCl_3$), sodium chloride, 2,2´-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2diphenyl-1-picrylhydrazyl (DPPH), $AlCl_3$, potassium acetate (CH_3CO_2K), vanillin, butylated hydroxyl toluene (BHT), ascorbic acid, n-butanol, diethyl ether, ammonia solution, ferric chloride ($FeCl_2$), acetone, methanol, ethanol, hydrochloric acid, sodium hydroxide, phosphate buffer, potassium ferricyanide ($K_3Fe(CN)_6$), ammonium molybdate, sodium phosphate, rutin, trichloroacetic acid (TCA), glacial acetic acid (CH_3COOH), and sodium nitroprusside ($Na_2[Fe(CN)_5NO]_2H_2O$). These chemicals were purchased from Merck and Sigma-Aldrich, Gauteng, South Africa. All the chemicals used in this study were of analytical grade.

In vitro quantitative phytochemical evaluation

Phenolic acid determination

Phenol determination was estimated spectrophotometrically using the Folin-Ciocalteu's method as described by Samatha *et al*.[7] with some modifications. Briefly, 0.5 mL of the plant extracts (1 mg/mL), standard gallic acid (0.02 mg/mL to 0.1 mg/mL), and the solvent of dissolution (control) was pipetted in different test tubes. To this, 2.5 mL Folin–Ciocalteu's reagent (10% [v/v]) was added. The mixture was vortexed. The reaction was left to stand at room temperature for about 5 min. Thereafter, 2 mL of anhydrous sodium carbonate (7.5%) was added to the solution, vortexed, and incubated at 40°C for 30 min. The control solution was used as a blank. After incubation, absorbance was read at 765 nm using a ultraviolet-visible 3000 PC spectrophotometer. The experiment was done in triplicate. The phenol content was extrapolated from the gallic acid standard/calibration graph equation; $y = 8.07 \times + 0.1$, R^2: 0.9981 and was expressed as mg gallic acid equivalent (GAE)/g from the equation CV/m; where "C" is the concentration as derived from the standard graph equation in mg/mL, "V" is the volume of the extract used in the assay in mL and "m" is the mass of the extract used in the assay in "g".

Flavonoid determination

The aluminum chloride colorimetric assay as described by Kamtekar *et al*.[8] with little modification was used to determine the flavonoid content of the different solvent extracts. The protocol is based on the quantification of the yellow-orange color produced when flavonoid reacts with $AlCl_3$. Briefly, 0.5 mL aliquots of the solvent fractions (1 mg/mL), different concentrations (0.2–1 mg/mL) of quercetin standard and the solvent of dissolution (control) were placed in different test tubes. Thereafter, 2 mL of distilled water was added to each test tube after which, 0.15 mL of 5% sodium nitrite was also added to the mixture. The mixture was left to stand for 6 min. After 6 min, 0.15 mL of $AlCl_3$ (10%) was added to the solution and left to stand for another 5 min thereafter 1 mL of 1 M sodium hydroxide was added. The solution was made up to 5 mL with distilled water, and the absorbance was read using a spectrophotometer at 420 nm blanking with the control solution. The experiment was done in triplicate. The flavonoid content was calculated using the calibration curve equation, $y = 1.064 \times$, $R^2 = 0.9976$ and expressed as mg of quercetin equivalent (QE)/g using the formula CV/m in the same manner as described in the phenolics above.

Proanthocyanidin (condensed tannin)

The protocol described by Oyedemi *et al*.[9] was used for the quantification of proanthocyanidin content of the samples. To 0.5 mL of the different solvent extracts (1 mg/mL), different concentrations (0.02 to 1 mg/mL) of the standard catechin and the solvent of dissolution (control), was added 3 mL of 4% (w/v) vanillin-methanol and 1.5 mL of hydrochloric

acid and the solution was vortexed. The solution was left to stand at room temperature for 15 min. Absorbance was read at 500 nm using the control solution as blank. The experiment was done in triplicate. Proanthocyanidin content was extrapolated using the calibration graph equation: Y = 0.9038 × + 0.0449, R^2 = 0.9951 and expressed as mg catechin equivalent (CE)/g using the formula, CV/m as earlier mentioned in phenol.

In vitro antioxidant analyses

The antioxidant activities of *P. capitata* were quantified using ABTS, reducing power, DPPH, nitric oxide, and phosphomolybdate (total antioxidant capacity [TAC]) assays.

(2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity assay

The method described by Kibiti and Afolayan[10] was used to evaluate the DPPH free radical scavenging activity of the samples. A solution of 0.135 mM DPPH in methanol was prepared. One mL of the DPPH solution was mixed with 1 mL (5 µg/mL to 80 µg/mL) each of the plant fraction/standard drug (BHT, Vitamin C). A control-containing DPPH solution and methanol only was also prepared. The reaction mixture was thoroughly vortexed and left to stand in the dark at room temperature for 30 min. Absorbance of the mixture was measured at 517 nm using a spectrophotometer blanked with methanol. The experiment was done in triplicate. The scavenging ability of the different plant fractions and standard was calculated using the equation:

DPPH Scavenging activity (%) = [(Absorbance of control– Absorbance of sample)/(Absorbance of control)] ×100

(2, 2'-Azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid) radical scavenging activity

The method described by Wintola and Afolayan[11] was adopted to investigate the ABTS radical scavenging activity of the different plant fractions. A working solution was first prepared by reacting 7 mM ABTS and 2.45 mM potassium persulfate in equal amounts (1:1) and allowed to stand for 12–18 h at room temperature in the dark to form a green-colored ABTS radical ($ABTS^+$). The resulting solution was further diluted by mixing 1 mL of the $ABTS^+$ solution with about 50 mL of methanol until an absorbance of 0.700 ± 0.006 at 734 nm is obtained. The $ABTS^+$ + methanol solution is adjusted by either adding the $ABTS^+$ if the absorbance were lower or adding methanol if the absorbance were higher than the stipulated unit. After obtaining the desired absorbance, 1 mL of the resultant solution is then mixed with 1 mL of the plant extract or standard drugs of different concentrations (5 µg/mL to 80 µg/mL) and left to stand in the dark for 7 min. A control solution as described in DPPH was also prepared. After 7 min, the reduction in absorbance was read at 734 nm using a spectrophotometer. The percentage inhibition of $ABTS^+$ by the different solvent extracts and standard drug was calculated using the equation:

ABTS Scavenging activity (%) = [(Absorbance of control– Absorbance of sample)/(Absorbance of control)] ×100.

Ferric reducing antioxidant power of the extracts

The ferric reducing antioxidant power (FRAP) of the different solvent fractions was assessed according to the method described by Aiyegoro and Okoh.[12] A mixture containing 2.5 mL of 0.2 M phosphate buffer pH 6.6 and 2.5 mL 1% potassium hexacyanoferrate was added to 1.0 mL of the different solvent extracts and standards (25 µg/mL to 400 µg/mL). The resulting mixture was incubated for 20 min at 50°C. After incubation, 2.5 mL of 10% TCA (w/v) was added to terminate the reaction and centrifuged for 10 min at 3000 rpm. 2.5 mL of the supernatant was removed and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% freshly prepared $FeCl_2$, $FeCl_3$, was added. The mixture was left to stand for 10 min, and the absorbance was read at 700 nm. A mixture of the buffer instead of the sample served as control. Increased absorbance of the reaction mixture indicated higher reducing power of the plant fractions. The percentage inhibition of the sample and the standard drug was calculated using the formula:

% inhibition = [(Absorbance of sample– Absorbance of control)/(Absorbance of sample)] ×100.

Nitric oxide scavenging activity

Briefly, 2 mL of 10 mM $Na_2[Fe(CN)_5NO]_2H_2O$ prepared in 0.5 mM phosphate buffer saline pH 7.4 was mixed with 0.5 mL of the different solvent plant fractions, Vit C and BHT (25 µg/mL to 400 µg/mL). The mixture was incubated at 25°C for 150 min thereafter 0.5 mL of the solution was mixed with 0.5 mL of Griess reagent [1 mL sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid) with 1 mL of naphthalene diamine dichloride (0.1% w/v) at room temperature for 5 min]. Finally, the mixture was incubated for 30 min at room temperature and the absorbance was read at 540 nm. A solution containing water instead of the extract/standard was used as a control.[11] The inhibited of nitric oxide by the different solvent fractions was calculated using the following equation:

(%) inhibition of NO = [(Absorbance of control– Absorbance of sample)/(Absorbance of control)] ×100.

Total antioxidant capacity (phosphomolybdenum assay)

The TAC of the plant fractions was determined by phosphomolybdenum method as described by Olugbami *et al.*[13] Briefly, 0.3 mL of the different solvent fractions and standard drugs (25 µg/mL to 400 µg/mL) were taken in test tubes and dissolved in 3 mL of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). The test tubes were covered and incubated at 95°C in a water bath for 95 min. The mixture was allowed to cool to room temperature and the absorbance was measured at 695 nm. A mixture containing distilled water instead of the samples served as control. Ascorbic acid and gallic acid were used as standard drugs. Higher absorbance indicates higher total antioxidant potential. The percentage inhibition was calculated as thus:

[(absorbance of sample– absorbance of control)/(absorbance of sample)] ×100

Statistical analysis

All the experiments were carried out in triplicates and data were expressed as mean ± standard deviation of three replications. Statistical analysis was performed by ANOVA. Where the data showed significance ($P < 0.05$), a mean separation was done using the Fisher's least significant difference with the aid of GENSTAT 8 statistical package VSN International Ltd, 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, Herts, UK HP1 1ES.

RESULTS

The resultant percentage yield after extraction with the various solvents was: 21.5% (64.5 g) for the methanol, 10.8% (32.5 g) for the water, 6.87% (20.6 g) for acetone, and 4.3% (12.9 g) for ethanol.

Phytochemical evaluation

Phenol content

The results of the phenolic content were expressed in mg of GAE per gram (mg GAE/g). The acetone extract had the highest phenol content of 218.62 ± 3.38 mg GAE/g while the aqueous extract had the lowest amount (175.53 ± 0.32 mg GAE/g). The methanolic and ethanolic

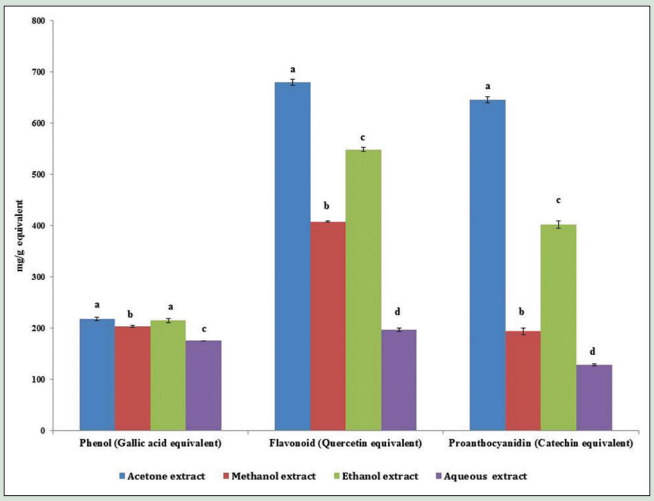

Figure 1: Phytochemical content of the different solvent fractions of *Phragmanthera capitata* in standard equivalents Values are mean ± standard deviation of three replications. Set of bars with different letters are significantly different ($P < 0.05$)

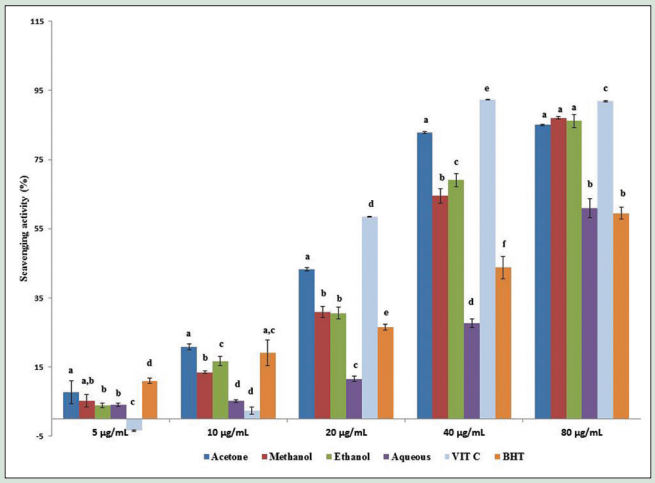

Figure 2: 2,2Diphenyl-1-picrylhydrazyl radical scavenging activity of the different solvent fractions of *Phragmanthera capitata*. Values are mean ± standard deviation of three replications. Set of bars with different letters are significantly different ($P < 0.05$)

extracts were 203.78 ± 1.95 mg GAE/g and 214.39 ± 4.09 mg GAE/g respectively. There was no significant difference between the acetone and ethanol extract ($P > 0.05$) [Figure 1].

Flavonoid content

The flavonoid content of the crude extracts was determined with reference to the standard quercetin and expressed as its equivalent (mg QE/g). The result of the evaluation also showed the acetone fraction to be significantly higher (679.82 ± 6.26 mg QE/g) than the rest solvent fractions ($P < 0.05$). Ethanol, methanolic, and aqueous fractions had values of 548.87 ± 4.10 mg QE/g, 407.90 ± 0.94 mg QE/g and 197.06 ± 3.80 mg QE/mg respectively. All the solvent fractions were significantly different from each other ($P < 0.05$) [Figure 1].

Proanthocyanidin (condensed tannin) content

The result of the proanthocyanidin was expressed as mg CE per gram (mg CE/g). Acetone extract had a significantly higher amount (645.68 ± 6.35 mg CE/g) than the rest solvent extracts ($P < 0.05$). The methanol, ethanol, and aqueous extracts were 194.00 ± 6.20 mg CE/g, 402.15 ± 7.55 mg CE/g, and 128.83 ± 1.69 mg CE/g, respectively. All the solvent fractions were significantly different from one another ($P < 0.05$) [Figure 1].

In vitro antioxidant compositions

2,2Diphenyl-1-picrylhydrazyl free radical scavenging assay

The DPPH radical scavenging activity of the different solvent extracts in comparison to known antioxidants (Vitamin C and BHT) and their respective concentrations that scavenged 50% (half maximal inhibitory concentration [IC_{50}]) of the radicals are presented in Figure 2 and Table 1, respectively. The scavenging activity of all the solvent fractions and standard drug increased with increase in concentration. At 40 µg/mL, almost all the solvent fractions had over 50% inhibitory activity (64.54 ± 2.09% to 82.73 ± 0.30%) on the DPPH radical except for the aqueous fraction with an activity of 27.59 ± 1.21% [Figure 2]. The IC_{50} (the ability of the fractions to scavenge or inhibit 50% of the radicals) ranged from 24.5 µg/mL in the acetone fraction to 67.2 µg/mL in the aqueous extract. The decreasing scavenging activity of the extracts and the standard drugs based on the IC_{50} was in the order; Vitamin C > acetone > ethanol > methanol > BHT > aqueous [Table 1].

2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assay

There was also a concentration-dependent response in this assay. All the solvent extracts showed great ABTS radical scavenging activity at very low concentrations. At the lowest concentration (5 µg/mL), the acetone and ethanol extracts had inhibitory activity on the ABTS + of over 50% (63.18 ± 8.19% and 58.61 ± 1.80%, respectively). At 10 µg/mL, all the solvent fractions and BHT had exceeded 60% scavenging activity. At 40 µg/mL, there was complete inhibition (100%) of all the solvent extracts and standard drugs [Figure 3]. The IC_{50} values ranged from <5 µg/mL in the acetone and ethanol fractions (1.9 µg/mL) and BHT (4.6 µg/mL), to 22 µg/mL in Vitamin C [Table 1]. The order of decreasing scavenging activity of the extracts and the standard drugs based on the IC_{50} is in the order; acetone ≥ ethanol > BHT > methanol > aqueous > Vitamin C.

Ferric reducing antioxidant power assay

The reducing power of the solvent extracts on ferric to ferrous gradually increased with increase in concentration. It was at the highest test concentration (400 µg/mL) that all the extracts had 50% and above reducing power why vit C had 50% reducing power at 100 µg/mL why BHT was not active in this assay [Figure 4]. The IC_{50} obtained for the solvent fractions and standard ranged from 89 µg/mL in Vitamin C to >>400 µg/mL in BHT [Table 1]. The increase in the reducing power activity of the solvent extracts and standard drugs as obtained from the IC_{50} is in the order; Vitamin C > methanol > ethanol > acetone > aqueous > BHT.

Nitric oxide scavenging assay

The result of the nitric Oxide scavenging activity of the different solvents showed that the acetone fraction had a scavenging activity which ranged from 46.41 ± 4.58% at the lowest concentration (25 µg/mL) to 73.15 ± 2.79% at 200 µg/mL. The methanol extract scavenging activity ranged from 64.99 ± 1.72% at the lowest concentration (25 µg/mL) to 75.26 ± 0.56% at 200 µg/mL. The ethanol scavenging activity ranged from 49.07 ± 0.43% at the lowest (25 µg/mL) concentration to 68.73 ± 3.95% at the highest concentration (400 µg/mL). Aqueous extract had an activity which ranged from 49.07 ± 2.56% at 25 µg/mL to 68.02 ± 4.32%

Table 1: IC_{50} values of the solvent fractions of *Pocillopora capitata* leaves and standard drugs

	DPPH		ABTS		FRAP		Nitric oxide		Phosphomolybdenum	
	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2
Acetone	24.5	0.9928	1.9*	0.8972	318	0.9677	23*	0.9796	85	0.998
Methanol	27.4	0.9585	5.7	0.9189	302	0.9748	1*	0.9773	124	0.974
Ethanol	26.4	0.9594	1.9*	0.7373	308	0.9423	41.9	0.981	87	0.979
Aqueous	67.2	0.9948	6.8	0.9551	374	0.9887	34	0.9727	144	0.994
Vitamin C	18.2	0.928	22	0.959	89	0.9906	10*	0.9999	18*	0.959
BHT	56	0.9661	4.6*	0.9966	NA	0.8942	27	0.9924	-	-
Gallic acid	-	-	-	-	-	-	-	-	2*	0.938

*Values are lower than the least concentration evaluated. IC_{50}: Concentration (µg/mL) required to scavenge/inhibit 50% of the radical; R^2: Coefficient of determination; Values obtained from regression lines with 95% confidence level; NA: Not active; -: Values not determined; DPPH: 2,2diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); FRAP: Ferric reducing antioxidant power

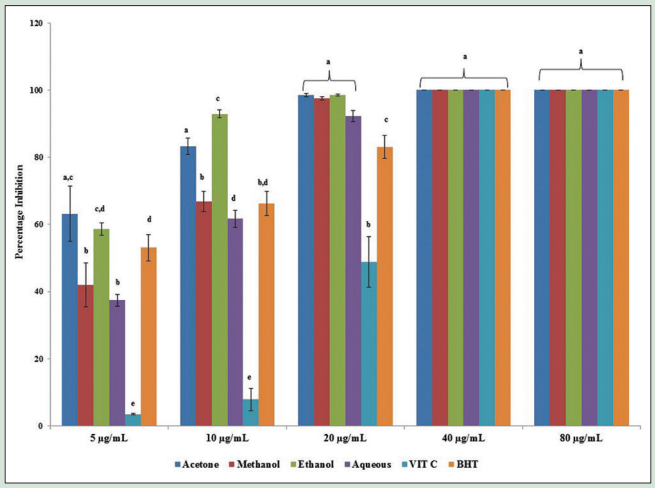

Figure 3: 2,2´-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of the different solvent fractions of *Phragmanthera capitata*. Values are mean ± standard deviation of three replications. Set of bars with different letters are significantly different ($P < 0.05$)

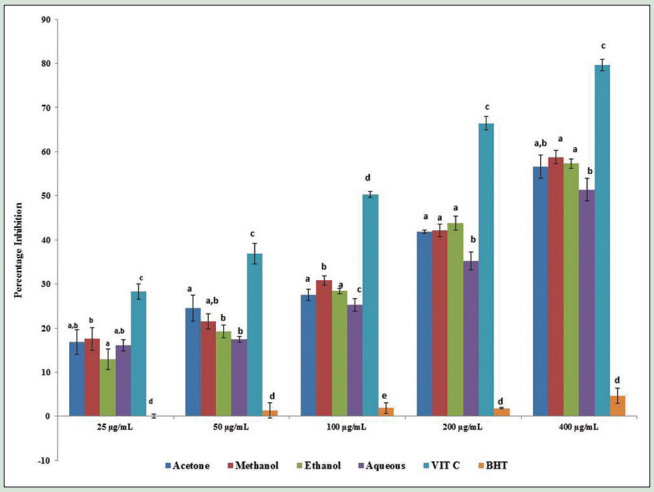

Figure 4: Ferric reducing the activity of the different solvent fractions of *Phragmanthera capitata*. Values are mean ± standard deviation of three replications. Set of bars with different letters are significantly different ($P < 0.05$)

at 400 µg/mL. The standard drugs, however, had their highest activities at 50 µg/mL (67.91 ± 2.97%) for Vitamin C and 100 µg/mL (60.292 ± 5.27%) for BHT [Figure 5]. The scavenging activity as recorded from the IC_{50} values is in the order; methanol > Vitamin C > acetone > BHT > aqueous > ethanol [Table 1].

Total antioxidant capacity

The TAC of *P. capitata* was measured by phosphomolybdenum method. The antioxidant capacity of all the solvent fractions increased with an increase in concentration. The acetone fraction ranged from 26.48 ± 2.12% at 25 µg/mL to 78.64 ± 0.77% at 400 µg/mL; methenaol extract fraction had a TAC from 19.66 ± 5.82% at 0.025 mg/mL to 75.49 ± 0.79% at 0.4 mg/mL; ethanol extract TAC was from 30.07 ± 2.47% at 25 µg/mL to 78.46 ± 0.12% at 400 µg/mL; and the aqueous extract TAC ranged from 12.27 ± 5.05% at 25 µg/mL to 70.30 ± 0.60% at 400 µg/mL [Figure 6]. The IC_{50} of the solvent fractions and the standard drugs in the order of decreasing TAC are gallic acid > Vitamin C > acetone > ethanol > methanol > aqueous [Table 1].

The relationship between the phytochemical contents and the IC_{50} of the antioxidant activities is as shown in Figure 7a-e. The phenol contents had a high linear correlation of $R^2 = 0.9228$ and 0.8658 based on DPPH and TAC assays, respectively. Flavonoid also had a high linear correlation with TAC ($R^2 = 0.9228$), but with DPPH, it showed a high-power correlation of $R^2 = 0.9098$. Proanthocyanidin content also had a high exponential correlation of $R^2 = 0.9405$ with TAC assay.

DISCUSSION

Phenols have been recorded to exhibit great antioxidant activities and this has been attributed to the presence of the hydrogen groups which acts as a hydrogen donor. Consequently, as a result of their redox property, they function as a reducing agent and act as radical scavengers.[11] The values of the phenol content in this study were evaluated based on the amount of GAE per gram in the sample. The values of the phenolic content obtained confirmed that the solvents had different abilities in extracting the phenols from the sample. The acetone fraction though not significantly different from the ethanol fraction ($P < 0.05$) extracted higher levels of the phenols in *P. capitata* at the same amount of evaluation. Worthy of note is that phenolic compounds are often associated with diverse biomolecules (proteins, polysaccharides, chlorophyll, terpenes, and inorganic compounds), and therefore, suitable solvent for the extraction of a particular class/group of compounds should be used based on the structural characters and related level of solubility of a target molecule.[14]

Like phenols, flavonoids are secondary metabolites with polyphenolic structure. They are also water soluble and have been reported to exhibit great antioxidant activities than Vitamins C, E, and carotenoids.[15] The flavonoid was estimated using quercetin as the reference. It was observed that the effect of the different solvents on the flavonoid content was similar to that of the phenol. The acetone fraction was significantly higher ($P < 0.05$) than the rest solvent extracts compared [Figure 1] and hence, forms the best solvent for the extraction of flavonoid from this sample. However,

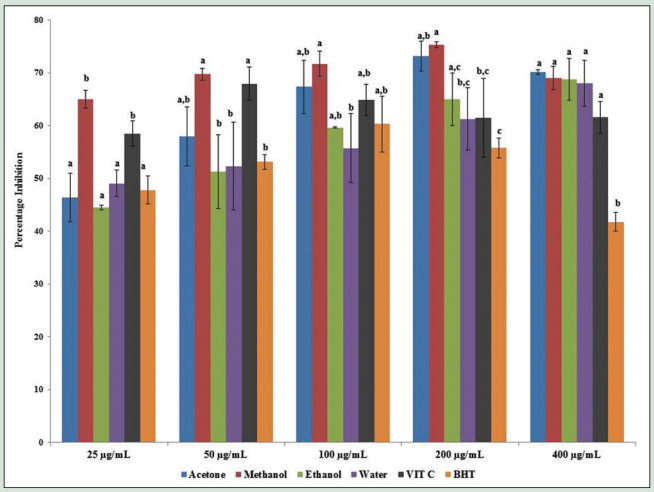

Figure 5: Nitric oxide scavenging activity of the different solvent fractions of *Phragmanthera capitata*. Values are mean ± standard deviation of three replications. Set of bars with different letters are significantly different ($P < 0.05$)

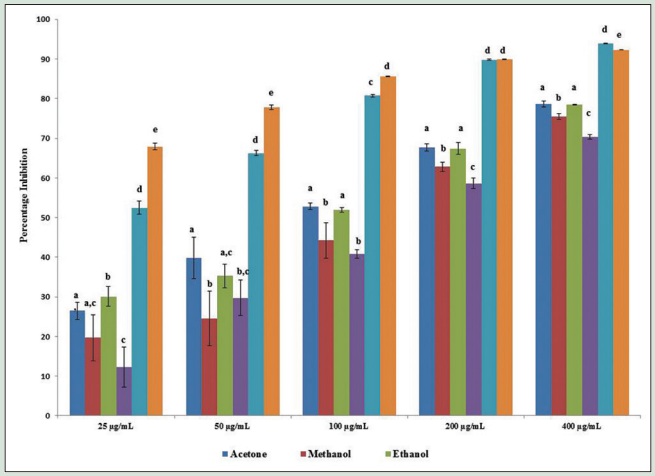

Figure 6: Total antioxidant capacity of the different solvent fractions of *Phragmanthera capitata*. Values are mean ± standard deviation of three replications. Set of bars with different letters are significantly different ($P < 0.05$)

water (aqueous) which is the cheapest solvent and readily available to all had the lowest quantity of flavonoid in relation to QE.

Condensed tannin also called proanthocyanidin is a group of polyphenolic bioflavonoids.[11] They are ubiquitous and present as the second most abundant natural phenolic after lignin. Proanthocyanidins are of great interest in nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health.[16] The result of this study showed that *P. capitata* is very rich in proanthocyanidin content in reference to catechin. Once again, the acetone extracts had a higher significant difference amount than the other solvent extracts ($P < 0.05$) and hence a solvent of choice for the extraction of proanthocyanidins.

Different *in vitro* methods were also employed to examine the effects of the acetone, methanol, ethanol, and aqueous extracts on the antioxidant activities of the sample. DPPH radical scavenging model is widely employed to evaluate antioxidant activities of natural compounds and plant extracts. The degree of discoloration from purple to light yellow indicates the scavenging potential of the extract which is a result of the hydrogen donating ability. This assay is very sensitive and can detect active ingredients at very low concentrations.[17] The result of this experimentation revealed that all the solvent fractions had great DPPH scavenging activities even at very low concentrations (5 µg/mL to 80 µg/mL). IC_{50} which is the concentration of the extract required to scavenge 50% of the radical is an indicator which reflects the activity of an extract. The lower the IC_{50} value, the higher the scavenging activity of the extract. In this study, all the solvent fractions exhibited strong DPPH radical scavenging abilities. The IC_{50} values for the acetone (24.5 µg/mL), methanol (27.4 µg/mL) and ethanol fractions (26.4 µg/mL), were very close to the standard Vit C (18.2 µg/mL) and lower than BHT (56 µg/mL). This result is in conformity with the results of the phytochemical analysis with a positive correlation of the polyphenolics (phenol, flavonoid, and proanthocyanidin) with DPPH and TAC. Solvent fractions with higher polyphenolics contents had lower IC_{50} values hence exhibited higher scavenging activity [Figure 7].

The ability of the solvent fractions to scavenge the ABTS radical was also evaluated. ABTS reacts with persulfate to form a bluish-green color (ABTS radical). When the ABTS radical formed reacts with a H-donor, it decolorizes to its neutral ABTS state. All the solvent fractions showed great scavenging activity on the ABTS radical at very low concentrations. The IC_{50} values of all the solvent fractions, which ranged from < 5 µg/mL to 6.8 µg/mL, were far lesser than the value for Vit C (22 µg/mL) [Table 1]. In this study, it was observed that the acetone and the ethanol extract had an IC_{50} lesser than the least concentration which indicates their greater effectiveness in scavenging ABTS radicals than the methanol and aqueous fractions.

The ferric reducing power of the solvent fractions was determined by the direct electron donation in the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The presence of the different solvent fractions caused a reduction of the Fe^{3+} complex to Fe^{2+} and was monitored at 700 nm. The antioxidant activity on FRAP was much lower than DPPH and ABTS as much higher concentrations were required to achieve the desired IC_{50} values for all the solvent extracts and standards used. The trend on the effects on FRAP was not directly proportional to the results of the polyphenolic contents of the plant as observed in DPPH and ABTS. The methanol fraction in this assay, had the strongest FRAP (IC_{50} = 302 µg/mL) followed by the ethanol (IC_{50} = 308 µg/mL), acetone (IC_{50} = 318 µg/mL), and aqueous (IC_{50} = 374 µg/mL), respectively [Table 1].

During the nitric oxide assay, $Na_2[Fe(CN)_5NO]_2H_2O$ decomposes in aqueous solution at physiological pH producing NO, making it an ideal assay to mimic the human body system in scavenging the free radical.[18] During this assay, nitrite is formed when NO generated from $Na_2[Fe(CN)_5NO]_2H_2O$ reacts with oxygen. Hence, it can be deduced that the plant fractions inhibit nitrite formation by directly competing with oxygen and other nitrogen oxides such as NO.[19] The IC_{50} values like in the FRAP, does not have a direct proportion to the polyphenolic compounds. The methanol fraction gave the best NO scavenging ability with an IC_{50} value lesser than the least concentration evaluated (<25 µg/mL) [Table 1].

The TAC of the solvent fractions was determined based on the reduction of molybdenum (VI) to molybdenum (V) and the subsequent formation of a green phosphate/molybdenum (V) complex at acidic pH. All the solvent fractions had a steady increase in TAC as the concentrations increased. At 100 µg/mL, the acetone and ethanol fractions had already reduced >50% molybdenum (VI) to molybdenum (V). Based on the $IC_{50,}$ the standard drugs (Vit C and gallic acid) were more potent than the solvent fractions with values far lesser than the lowest concentration used for this assay. However, the effect of the different solvents showed acetone to be more potent with an IC_{50} value of 84 µg/mL followed by

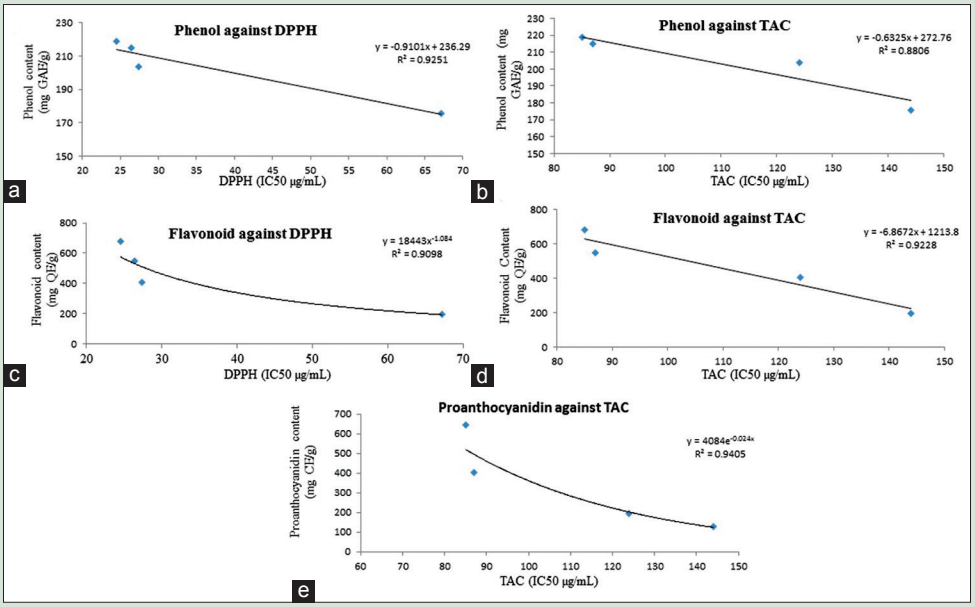

Figure 7: Correlation of the polyphenolic compounds against the IC50 of the antioxidant (a-b) Phenol against DPPH and TAC respectively, (c-d) Flavonoid against DPPH and TAC respectively and (e) Proanthocyanidin against TAC

the ethanol fraction with a value of 87 µg/mL. Methanol and aqueous fractions showed lesser TAC values with IC_{50} values of 124 µg/mL and 144 µg/mL, respectively. There was a positive correlation of the polyphenolic content with the IC_{50} values of TAC.

CONCLUSION

This study revealed the effect of different solvents on the extraction and activities of *P. capitata* harvested from rubber tree. From the result of this findings, if the work were just to extract components for preparatory work only without bioassay, methanol would be the solvent of choice as it gave better yield than the rest solvents. If the intention were for activity screening, acetone will be preferred as it had the highest polyphenols and antioxidant activities.

Acetone has the ability to dissolve both hydrophilic and lipophilic compounds and is miscible with water, less volatile, and a very useful extractant in bioassays. It is easy to handle at different stages of bioassays and preferred to methanol, ethanol, and water even when more hydrophilic components are investigated. This is because acetone extracts highly polar components and a very useful extractant for dried plant materials.[20]

The results of this experimentation showed that *P. capitata* has high amounts of polyphenolic compounds and in turn, exhibited great antioxidant activities in all the solvent fractions tested. This plant may have great importance in combating oxidative stresses and hence, expanding its application in health delivery is recommended.

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Conflicts of interest

There are no conflicts of interest.

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