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Determination of the Total Phenolic Contents, Antimicrobial and Antioxidant Effects in the Ethanolic Leaf Extract of *Prunus Amgydalus*

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ABSTRACT

The results showed that the maximum yield of flavonoids (26.54 mg/ml quercetin equivalent) can be obtained with 80% ethanol (v/v), extraction temperature of 80^{o}C and raw material to solvent ratio of 0.048 g/ml. The results revealed presence of flavonoids, saponins, alkaloids and glycosides. The leaf extract was active against *Pseudomonas aeruginosa*, and *Staphylococcus aureus* with MIC of 0.05 mg/ml while it was inactive against *Pseudomonas selanacea, Fusarium oxysporum, Kliebsella pnuemoniae, Escherichia coli, Bacillus subtilis, Bacillus cereus* and *Candida albacans.* The total phenolic and flavonoid contents obtained were 40.35 mg/ml Gallic acid equivalent and 26. 54 mg/ml quercetin equivalent while the antioxidant assay showed a concentration dependent antiradical activity resulting from reduction of DPPH, SO and OH radicals to non radical forms. The scavenging activity of the ascorbic acid was seen to be higher than that of the extract.

INTRODUCTION

About 80% of the world's inhabitants rely mainly on traditional medicine which involves the use of plants for their primary health care (Chan *et al.,* 2006; Owolabi *et al.,* 2007). The use of herbal medicine has increased over the years. Moreover, pharmaceutical companies in the developed societies have developed methods of extraction and development of drugs chemotherapeutics from medicinal plants (WHO, 1997). World health Organization has recognized that medicinal plants are good sources of drugs and therefore, should be investigated in order to understand their properties, safety and efficacy (Nacimento *et al.,* 2000). The plant *Prunus amygdalus* commonly known as almond or umbrella tree belongs to the family of plants known as Rosaceae. It is found in the east Mediterranean countries, Africa and Australia. It is a deciduous tree and grows up to the height of 10.0 m with a tree trunk width of about 30 cm. The flowers are either white or pink in colour and are about 3.5-5 cm wide with five petals produced singly or in pairs. The leaves are 3-5 cm long (Josse *et al.,* 2007), with a serrated margin and a petiole which is about 2.5 cm long.

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The almond is popular because of the edible drupe which is also referred to as the fruit or nut. Most studies on this plant have been on the nut. Compounds that have been isolated from the nut include amygadaxin and sphingolipids (Spiller *et al.,* 2003); while flavonoids were isolated from the skin of the drupe (Lamarche *et al.,* 2004).

A prenylated benzoic acid derivative and some flavonoids were isolated from the hulls of the nut of *Prunus amygdalus*. It was shown that the sphingolipids isolated from the nuts were capable of inhibiting the development of colon cancer and also decrease the proportion of adenocarcinomas in mice. The leaves were found to possess some measure of protection against colon cancer. Based on the ethnopharmacological aspects, locally, in western Nigeria, the leaves are infused in hot water and taken as tea to induce sleep. Despite the extensive use of the plants by Nigerians, there have been limited attempts to explore the biological properties of the leaves of *Prunus amaygalus* in relation to their medicinal applications. This research work was undertaken in order to determine the phytochemicals present in the leaves, the antimicrobial activity, total phenolic and flavonoid contents and antioxidant activity of the ethanolic leaf extract of *Prunus amygdalus* that grows in Ogbomoso, Nigeria.

MATERIALS AND METHODS

Folin-Ciocalteau reagent and other chemicals used were of analytical grade and were Merck products. 1, 1-diphenyl-2picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO. USA). Genesys 10S vl. 200 2l7H311008 spectrophotometer was used for absorbance measurements.

Sample collection and preparation

The plant leaves of Prunus amygdalus was collected from Ladoke Akintola University of Technology, Ogbomoso, Nigeria. It was identified in the Department of Pure and Applied Biology by the botanist, Mrs. F. A. Ogundola. The plant leaves were air dried in the laboratory for two weeks, pulverized into fine powder and stored in airtight bottles awaiting further use.

Extraction

In order to determine the maximum flavonoid content in the extract, proper ranges of ethanol concentration (65-90%), extraction temperature (35-90^{o}C), raw material to solvent ratio (0.016-0.048), and time of extraction (1-9 hr) were preliminarily determined. A three level and three variables were used to determine the best combination of the extraction variables for the maximum production of flavonoids from the leaf of *Prunus amygdalus*. The independent factors and the dependent variables used are shown in table 1. The extraction was carried out using a Soxhlet extractor. Thereafter, the extracts were filtered and centrifuged at 5000 rpm for 15 min. The supernatants were concentrated using a rotary evaporator at room temperature and transferred into 100 ml beaker. It was left on laboratory bench for 5 days to dry properly which resulted in dark green extracts (Sasikumar *et al.,* 2005). The extracts were subjected to spectrophotometric analysis in order to quantify the flavonoids present in the extract.

Table. 1: Factors and levels used in the ethanol extraction of flavonoids from the leaves of *Prunus amygdalus.*

Factors	Levels		
	1	2	3
A – extraction temperature (^{o}C)	35	80	90
B – ethanol concentration (%)	65	80	90
C – raw material to solvent ratio (g/ml)	0.016	0.032	0.048

Phytochemical studies of the ethanolic extract using thin layer chromatography (tlc)

An attempt was made to determine the presence or absence of certain phytochemicals in the ethanolic leaf extract using the method described by Tona *et al.,* (1998). The presence of alkaloids was determined by using 10 g of the ethanolic extract of *Prunus amygdalus*. It was wetted with a half diluted NH_4OH and lixiviated with EtOAC for 24 hrs at room temperature. The organic phase was separated and basified with NH_4OH (pH 11-12), then extracted with chloroform (3x). The chloroform extract was concentrated using a rotary evaporator. The extract obtained was then used for tlc using the solvent mixture chloroform and methanol (15:1). The tlc plates were sprayed with Dragendorff's reagent and viewed under UV/visible light (254 nm). The R_f value of the separated alkaloids were recorded. To determine the presence of flavonoids, 10 g of the crude extract of *Prunus amygdalus* was suspended in distilled water and extracted with EtOAc. The mixture was separated using a separating funnel. The EtOAc portion was concentrated in a rotary evaporator and the extract used for tlc. The components were separated using chloroform and methanol solvent mixture (19:1). The tlc plate was sprayed with 10% NaOH. The colour and R_f values of the spots were recorded under UV light (275 nm). For the determination of presence of polyphenolics, 10 g of the crude extract of *Prunus amygdalus* was used for TLC by using the solvent mixture chloroform and methanol (27:1). The developed plate was sprayed with Folin-Ciocalteau's regent and placed in the oven for 10 min at a temperature of 80^{o}C. The R_f value was recorded under UV light (366 nm). The presence of saponins was evaluated using 10 g of the crude extract of *Prunus amygdalus* which was suspended in distilled water and extracted with n-butanol. The n-butanol portion was used for tlc.

The solvent mixture used was chloroform, glacial acetic acid, methanol and distilled water (64:34:12:8). The developed plate was placed in an iodine tank for visualization. The R_f values were obtained and recorded. The presence of glycosides was also evaluated by using 10 g of the crude extract of *Prunus amygdalus.* This was dissolved in 100 ml of ethanol and filtered with Whatman Number 1 filter paper. 70% of lead acetate was added to the filtrate and centrifuged at 5000 rpm/10 mins. 6.3% sodium carbonate was added to the supernatant and further centrifuged at 10000 rpm/10 mins. The supernatant was collected, dried and redissolved in chloroform and used for tlc with a solvent mixture of EtOAc, methanol and distilled water (80:10:10). The plate was sprayed with Fehling's solution and viewed under UV light. The R_f values were obtained. The presence of steroids was determined using 10 g of the crude extract of *Prunus amygdalus.* This was subjected to tlc using the solvent mixture chloroform, glacial acetic acid, methanol and distilled water (64:34:12: 8). The developed plate was sprayed with anaisaldehyde-sulphuric acid reagent. The plate was heated at 100 ^{o}C for 6 mins, cooled and the R_f values obtained under UV light.

Determination of R_f values

The distance travelled by the solvent (solvent front) and the components were measured and used to calculate the R_f values of the components.

Antimicrobial studies

Microbial strains

The microorganisms (*Pseudomonas selanacea, Pseudomonas aeruginosa, Staphylococcus aureus, Fusarium oxysporum, Kliebsella pnuemoniae, Escherichia coli, Bacillus subtilis, Bacillus cereus* and *Candida albacans*) used were clinical isolates collected from Baptist Medical Center, Ogbomoso, Nigeria.

Antibacterial activity

An inoculum of each of the bacterial strain (single colony) was suspended in 5 ml of broth (nutrient broth) and incubated at 37°C for 18 hr. The antibacterial activity was tested by the disc diffusion assay of Bauer *et al.,* 1996. The bacterial cells were harvested by centrifuging at 5000rpm for 15 min. The pellet formed was washed twice with PBS and the cells were counted by haemocytometer.

The bacterial cells were diluted to approximately 10^5 CFU/ml before use. 0.1ml of the inoculum (10^5 CFU/ml) was spread on sterile Mueller Hinton plates and sterile paper discs were placed on the inoculated surface. The discs were impregnated with 15µl of the extract at two different concentrations (100 and 200mg/ml), kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24 hr. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition as per the procedure described by Kim *et al.,* 1995. Ciprofloxacin a standard drug was used simultaneously as control.

Minimum Inhibitory Concentration (MIC)

1 ml of broth medium was taken into 10 test tubes. Different concentrations of plant leaf extract ranging from 0.125 to 8 mg/ml were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculum of test strains (10^5 CFU/ml) and kept at 37°C for 24 hr. The test tube containing the lowest concentration of extract which showed reduction in turbidity, when compared with control was regarded as MIC of that extract.

Determination of the antioxidant property of the ethanolic leaf extract

DPPH Radical scavenging activity

Different concentrations of the ethanolic extract were prepared and mixed separately with 0.1 mM solution of 2, 2-diphenyl-1-picrylhydrazyl in methanol. The mixture was incubated at room temperature for 30 min in the dark and later placed in a visible spectrophotometer.

The absorbance reading was obtained at 517 nm. Ascorbic acid was used as the antioxidant standard. The free radical scavenging activity of the sample was calculated using the formula:

$[(A_o - A_1)/A_o] \times 100\%$

Where

A_o is the absorbance of the control, while A_1 was the absorbance obtained from the sample (Gulcin, 2010).

Super oxide radical scavenging activity

The method used is that described by Nishimiki *et al.,* (1972). To a mixture of phosphate buffer (100mM, pH = 7.4), nitroblue tetrazolium (NBT) (1mM), NADH (1mM) and 50 µg/ml of extract in methanol was added 1 ml of 1M Mphenazine methosulfate. This mixture was incubated for 5 min at 25°C. The absorbance was measured at 560 nm against a blank, while ascorbic acid was used as the positive control.

Hydroxyl radical scavenging activity

To a mixture containing 0.4 ml of 20mM sodium phosphate buffer (pH = 7.4), 0.1 ml of mM deoxyribose, 0.1 ml of 10mM H_2O_2, 0.1 ml of mM ferric chloride and 0.1 ml of 1 Mm EDTA was added 0.1 ml of 2mM ascorbic acid and 0.1ml of 50 µg/ml of the extract. This mixture was incubated at 37 °C for 1 hr. Then, 1 ml of 17 mM each of 2- thiobarbituric acid and trichloroacetic acid were added. This mixture was boiled for 15 min and later cooled in ice. The absorbance was measured at 532 nm. Ascorbic acid was used as the positive control while the extract without deoxyribose was used as the blank (Halliwell *et al.,* 1987).

Determination of the total phenolic content of the ethanolic extract

0.5 ml of the extract was mixed with 2.5 ml of Folin-Ciocalteau reagent and 2.0 ml of 7.5% (w/v) Na_2CO_3. The mixture was vortexed and incubated at room temperature for 90 min. The mixture was placed in a visible spectrophotometer and the absorbance was obtained at wavelength of 765 nm. The result was expressed as mg Gallic acid equivalents/g extract (Ismail *et al.,* 2010).

Determination of the total flavonoid content of the ethanolic extract

0.1ml of extract was mixed with 0.3 ml of 5% (w/v) $NaNO_2$. This was incubated for 5 min. Later, 2 ml of 1 N NaOH and 0.3 ml 10% (w/v) of $AlCl_3$ were added to the mixture and made up to 5 min with distilled water. The total mixture was placed in a visible spectrophotometer and the absorbance reading taken at a wavelength of 420 nm. The results were expressed as mg Quercetin equivalents/g extract (Tsai *et al.,* 2007).

RESULTS

Effect of extraction time on the yield of flavonoids

The extraction time was set between 1 - 10 hrs in order to examine the influence of extraction time on the flavonoids yield when other extraction conditions were as follows: extraction temperature 80 °C, concentration of ethanol 80 % and the raw material to solvent ratio is 25:1. Fig. 2 shows the effect of extraction time on the yield of flavonoids from the leaf of *Prunus amygdalus*. The results show that the yield increased with increase in time up to 6 hrs and remained stable. The maximum yield obtained was 26.54 mg/g. Therefore, the optimum extraction time is considered to be 6 hrs.

Effect of raw material to solvent ratio on the yield of flavonoids

The raw material to solvent ratio used was 0.008, 0.016, 0.024, 0.032, 0.04 and 0.048 g/ml while the extraction time was set at 6 hrs, concentration of the extraction solvent was 80% and extraction temperature was 80 °C. The yield of the extracted flavonoids increased gradually as the ratio increased from 0.016 –

0.048 g/ml and then stabilized. This shows that the highest yield (23.86 mg/g) will be obtained with solvent to raw material ratio at a maximum of 0.048 g/ml (Fig. 2).

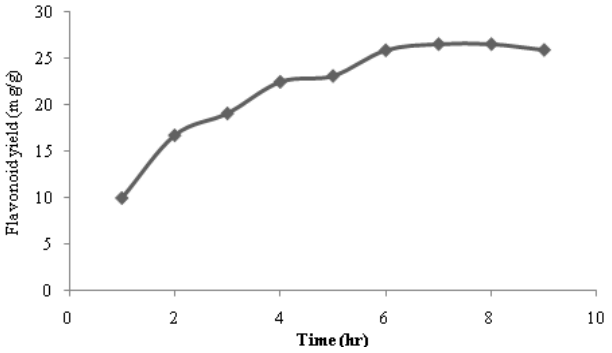

Fig. 1: Effect of extraction time on yield of flavonoids.

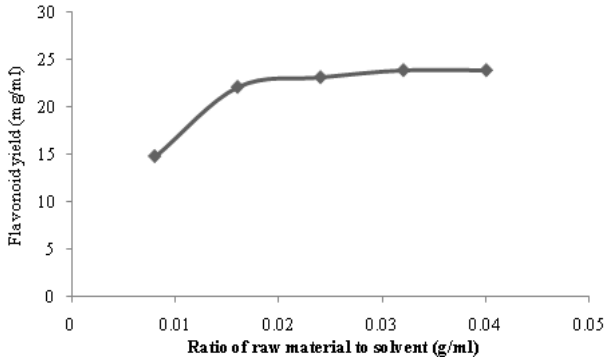

Fig. 2: Effect of ratio of raw material to solvent on yield of flavonoid.

Effect of extraction temperature on the yield of flavonoids

The influence of the extraction temperature was determined when the other extraction parameters were set as follows: ethanol concentration (80%), extraction time (6 hrs) and solvent to raw material ratio was 25:1. A sharp increase in yield was observed as the temperature increased to 80^{o}C. No further increase was observed after this temperature. The maximum yield obtained was 4.7 mg/g (Fig. 3).

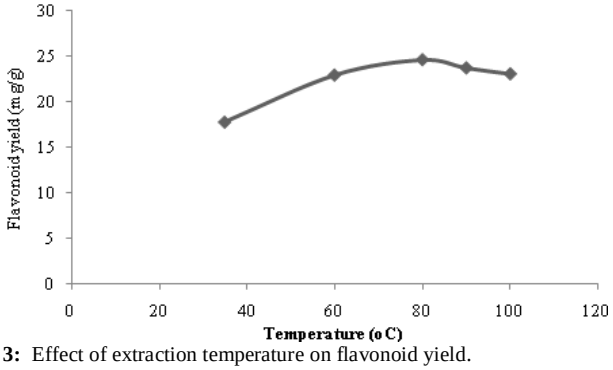

Fig. 3: Effect of extraction temperature on flavonoid yield.

Effect of concentration of extraction solvent on the flavonoid yield

Various concentrations of ethanol (65%, 80%, 90%) were used to determine the effect of extraction solvent on yield of flavonoids when other extraction parameters were set at 6 hrs extraction time, 20:1 solvent to raw material ratio and extraction temperature was 80 ^{o}C. A sharp increase was observed from 20-80 ^{o}C. The maximum yield of flavonoids (24.68 mg/g) was obtained at a concentration of 80 ^{o}C (Fig. 4).

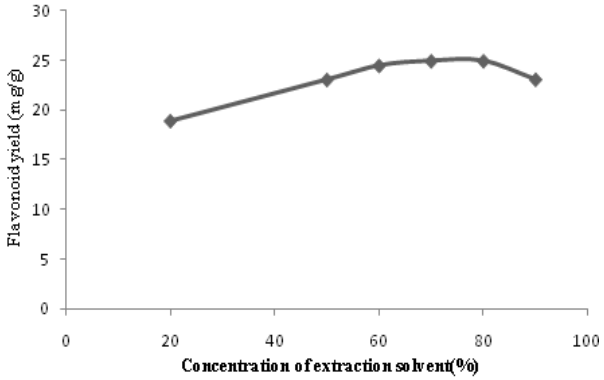

Fig. 4: Effect of extraction solvent concentration on yield of flavonoid.

Phytochemical studies

Preliminary phytochemical investigation revealed the presence of flavonoids, saponins, tannins and glycosides in the ethanol extract as shown in table 2.

The tlc analysis revealed presence of 3 spots each for flavonoids and alkaloids, 2 spots for saponins and 4 spots for glycosides. Their R_f values are given in table 2.

Table. 2: Phytochemical analysis of the crude extract.

Phytochemicals	fla	Ste	Tan	Alk	Sap	Gly
Availability	+	-	-	+	+	+
R_f values	0.61 0.77 0.84	-	-	0.44 0.59 0.73	0.87 0.96	0.67 0.71 0.83 0.89
No. of spots	3	-	-	3	2	4

Note: + = Present; - = Absent; fla -= flavonoids, Ste = Steroids; alk = alkaloids; Tan = Tannins; Sap = Saponins;Gly = Glycosides

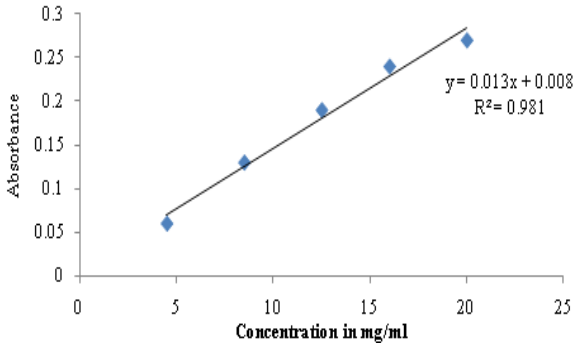

Fig. 5: Standard curve for Quercetin.

Antimicrobial activity

The data on the antimicrobial activity of the ethanolic extract of *Prunus amagydalus* is shown in Table 3.

The extract was effective against the screened Gram-positive bacteria and inactive against the Gram-negative bacteria and fungi.

Table. 3: Antimicrobial activity of crude ethanolic extract.

Conc (mg/ml)	ZONES OF INHIBITION OF MICROORGANISMS								
	EC	*PS*	*SA*	*FO*	*BS*	*PA*	*BC*	*CA*	*KP*
0.05	4±0.01	-	17±0.02	-	8±0.01	15±0.01	5±0.01	-	-
1.0	6±0.01	-	16±0.01	-	8±0.01	15±0.01	8±0.01	-	-
1.5	8±0.01	-	20±0/02	-	10±0.01	15±0.01	12±0.01	-	-
2.0	12±0.01	-	24±0.02	-	10±0.01	15±0.01	12±0.01	-	-

NOTE: - = No Zone of inhibition; EC - *E. Coli;* PS - *P selanacea; SA- S. aureus; FO- F. oxysporum;BS - B. subtilis; PA - P. aeruginosa; BC - B cereus; CA - C. albacans; KP - K. pneumonia*

Total phenolic and flavonoid contents

The phenolic content of the ethanolic leaf extract of *Prunus amygdalus* was determined from a linear gallic acid standard curve (y = 0.023x + 0.292, R^2 = 0.946) (fig.6). The total phenolic content obtained was 40.35 mg gallic acid equivalent/g extract. In this study the total flavonoid content of the ethanolic leaf extract was evaluated by using the aluminium colorimetric assay. Quercetin was used as the standard and the total flavonoid content was determined from the quercetin standard curve (y = 0.013x + 0.008, R^2 = 0.981) (fig. 7) and the results obtained expressed in mg quercetin equivalent/g extract (26.54 mg quercetin equivalent/g extract). Table 4 shows the total phenolic and flavonoid contents of the ethanolic leaf extract of *Prunus amygdalus.*

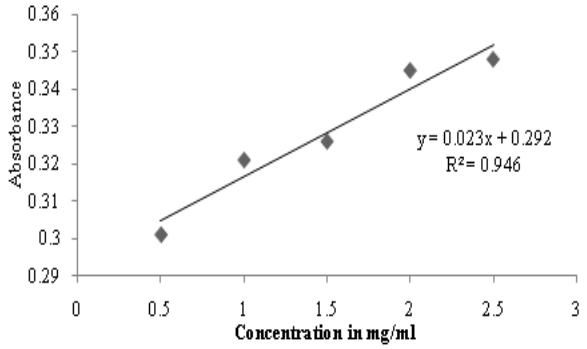

Fig. 6: Standard curve for Gallic acid.

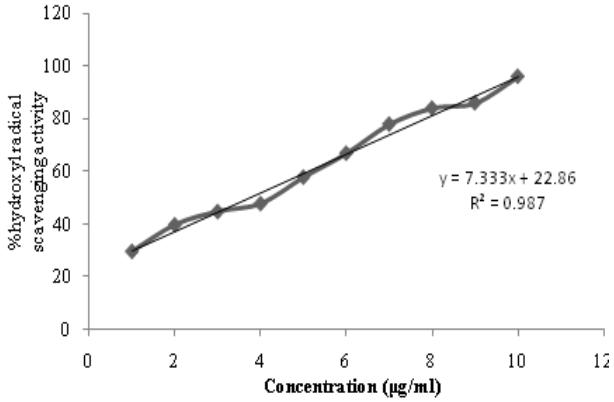

Fig. 7: Inhibition of hydroxyl radical by ascorbic acid.

Table. 4: Total phenolic and flavonoid contents of the methanolic leaf extract of *Prunus amygdalus* .

Parameter	Values
Total phenolic content (mg/ml gallic acid equivalent)	40.35
Total flavonoid content mg/ml quercetin equivalent)	26..54

In-vitro antioxidant assay

The analysis of the free radical scavenging activity of the ethanolic leaf extract resulted in the reduction of the DPPH, hydroxyl and superoxide radicals to the non radical form. The scavenging activity of ascorbic acid was used as the positive control. The scavenging activity of the extract was seen to be lower than that of the standard (Figs. 8-13).

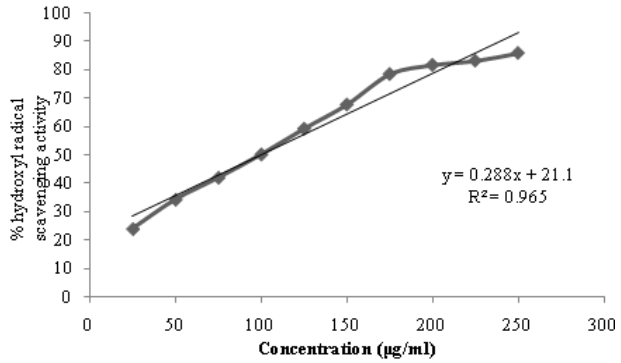

Fig. 8: Inhibition of hydroxyl radical by leaf extract.

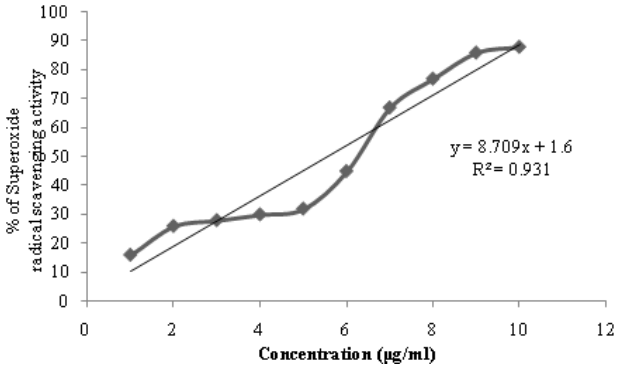

Fig. 9: Inhibition of superoxide radical by Ascorbic acid.

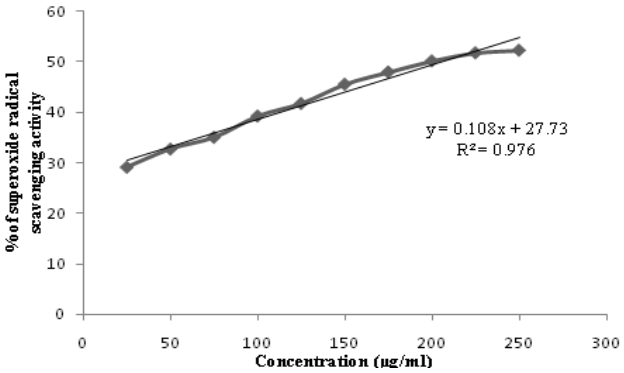

Fig. 10: Inhibition of superoxide radical by leaf extract.

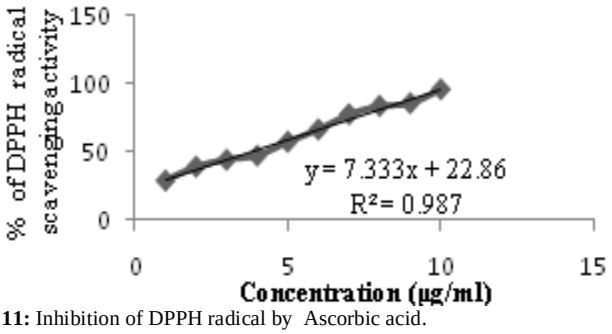

Fig. 11: Inhibition of DPPH radical by Ascorbic acid.

DISCUSSION

On the basis of single factor experiment using the relationships described in Table 1, the results show that the highest yield was obtained when the extraction temperature was 80 ^{o}C. This shows that temperature has a profound effect on the yield of flavonoids. As the temperature increased a decline in the yield was observed. This could be as a result of decomposition of the flavonoids at higher temperatures. The optimum conditions for the extraction of flavonoids was observed to be as follows: extraction solvent concentration (ethanol, 80%), extraction temperature 80 ^{o}C and raw material to solvent ratio 25 g/ ml. This study has shown the appropriate extraction conditions for flavonoids from the leaves of *Prunus amygdalus* and validated the flavonoid contents; thereby obtaining an informative profile which may serve as a basis for the utilization of the leaves of the plant. The presence of flavonoids, saponins, tannins and glycosides in the ethanol extract could be responsible for the medicinal properties of the leaves of the plant.

This study will aid the isolation and quantitative estimation of the pharmacologically active compounds. These data have provided chemical basis for the wide use of the plant leaves as therapeutic agents and for future probing of bioactive compounds from the leaves of *Prunus amygdalus*. The effectiveness of the extract on only Gram-positive bacteria could be as a result of their morphological differences with regard to their cell composition. The Gram-positive bacteria possess an outer peptidoglycan layer which is not an effective permeability barrier whereas the Gram-negative bacteria have outer polysaccharide membranes that contain the lipopolysaccharide components. It can also be attributed to the physico chemical properties of the bioactive principles present in the ethanolic leaf extract. Scientists have indicated that Phenolics and flavonoids have several biological properties such as antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial in humans (Havsteen, 1983; De Groot, 1994). The results obtained show that the high level of antioxidant activity was due to the presence of phenolic compounds in the leaves. These compounds are considered to be important plant constituents because of their scavenging ability which is as a result of the presence of hydroxyl groups. These compounds retard oxidative degradation of lipids thereby improving the quality and nutritional value of food (Meda *et al.,* 2005). Antioxidants are substances which can inhibit an oxidation process. The level of antioxidant activity of the leaves of *Prunus amygdalus* was determined by using DPPH in methanol. The free radical scavenging activity of the ethanolic leaf extract was compared with ascorbic acid as standard. It showed the dose dependent variation as expected. It exhibited strong antioxidant activity in methylated DPPH. The hydroxyl radical which is a very reactive species that is formed in biological systems has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu *et al.,* 2001). The effect of the extract on the inhibition of free radical-mediated deoxyribose damage was assessed by means of the iron (II) dependent DNA damage assay. The Fentone reaction generates hydroxyl radicals which degrade the DNA deoxyribose, using iron (II) salts as catalyst. The leaf extract was capable of reducing DNA damage at all concentrations used. This shows that the extract exhibited potential inhibitory effect of the hydroxyl radical scavenging activity. The superoxide is another reactive oxygen species that can cause damage to living cells and DNA leading to various diseases. In this study superoxide radicals were generated by auto-oxidation of hydroxylamine in the presence of NBT. The reduction of NBT in the presence of antioxidants was measured. Fig. 11 shows that the extract had a dose dependent scavenging activity on the superoxide radicals. But when compared to ascorbic acid it was found to be lower. This shows that the plant leaf extract contains constituents which could protect cells against lipid peroxidation and the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. These species are produced in an organism during oxygen metabolic reactions or are induced by exogenous damage (Coudert *et al.,* 1994) that contributes to the development and maintenance of cellular life (Nijveldt *et al.,* 2007). Due to reactions of these free radicals with endogenous molecules such as DNA, proteins and lipids, the body cells and tissues are continuously threatened by damage. Flavonoids have been found to have an additive effect to endogenous scavenging compounds and can interfere with three or more different free radical producing systems. Korina and Afanas'ev observed that flavonoids can prevent injuries caused by free radical by reacting with the reactive compound of the radical. This was achieved due to the high reactivity of the hydroxyl groups on the flavonoids. By directly scavenging radicals flavonoids can inhibit LDL oxidation in vitro thereby protecting the LDL particles which could result in the prevention of atherosclerosis (Kerry and Abbey, 1997). The antioxidant activity of the extract could be due to the presence of flavonoids in the leaves of *Prunus amygadalus.*

CONCLUSION

The present study revealed the optimum conditions for the extraction of flavonoids from the leaves of *Prunus amagydalus* and showed that significant amount of phytochemicals such as

flavonoids, saponins, tannins and glycosides are present in the plant leaves. The flavonoids in particular contain broad spectrum of chemical and biological activities including radical scavenging properties. Therefore, this plant leaves can be used as a natural source for antioxidant.

The presence of these phytochemicals could be responsible for its antibacterial activity.

REFERENCES

Bauer AW, Kirby WMM, Sherris JC. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1996; 45: 493 - 496.

Chan KF, Eze CA, Emuelosi CO. Antibacterial and wound healing properties o some Nigerian Medicinal plants. J. Ethnopharmacol. 2006; 104, 164-167.

Coudert PC, Rubat C, Sautou V, Bastide M, Chopineau J. The free radicals. *Actualités* Pharmaceutiques. 1994; 321: 37-46.

De Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology, 1994; 41: 328- 332.

Gulcin I. Antioxidant properties of resveratrol; A structure-activity insight. *Innovative Food* Science and Emerging Technologies. 2010; 11:210-218.

Havsteen B. Flavonoids, A class of natural products of high pharmacological potency. Biochem Pharmacol. 1983; .32: 1141–1148.

Ismail HI, Chan KW, Mariod AA, Ismail M. Phenolic content and antioxidant activity of cantaloupe (C*ucumis melo*) methanolic extracts. Food Chemistry. 2010; 11: 643-647.

Josse AR, Kendall CW, Augustin LS, Ellis PR, Jenkins DJ. Almonds and postprandial glycemia - a dose-response study. Metabolism. 2007; 56 (3): 400-404.

Kerry NL, Abbey M. Red wine and fractionated phenolic compouns prepared from red wine inhibits low density lipoprotein oxidation in vitro**.** Atherosclerosis. 1997; 135: 93-102.

Kim J, Marshall MR, Wei C. Antibacterial activity of some essential oil components against five food borne pathogens**.** J. Agri. Food Chem.1995; 43: 2839–45.

Korkina LG, Afanas'ev IB. Antioxidant and chelating properties of flavonoids. *Adv.* Pharmacol. 1997; 151-163.

Lamarche B, Desroches S, Jenkins DJ. Combined effects of a dietary portfolio of plant sterols, vegetable protein, viscous fibre and almonds on LDL particle size. Br. J. Nutr. 2004; 92 (4):657-63.

Meda A, Lamien CE, Romito M, Milloo J, Nacoulma OG. Determination of the total phenoic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity, Food Chem. 2005; 91: 571-577.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz. J. Microbiol. 2000, 31: 247-256.

Nijveldt RJ, Van Nood E, Van Hoorn DEC, Boelens PG, Van Norren K, Van Leeuwen PAM. Flavonoids: A review of probable mechanisms of action and potential applications. American J. of Clinical Nutrition. 2007; 74 (4), 418-425.

Owolabi OJ, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella africana* (Bignoniaceae) stem bark. *Afr. J.* Biotechnol. 2007;.6: 1677-1680.

Sasikumar JM, Pichai A, Doss A. Antibacterial activity of *Eupatorium glandulosum* leaves**.** Fitoterapia. 2005; 76 (2): 240-243.

Spiller GA, Miller A, Olivera K. Effects of plant-based diets high in raw or roasted almonds, or roasted almond butter on serum lipoproteins in humans. J Am Coll Nutr. 2003; 22 (3): 195-200.

Tsai P-J, Tsai, T-H, Yu C-H, Ho S-C. Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of green tea. Food Chemistry. 2007;.103: 181-187.

Tona L, Kambu K, Ngimbi N, Cianga K, Vlietnick AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol., 1998; 61:57-65.

WHO Resolution-Promotion and Development of Training and Research in Traditional Medicine. WHO Document. 1997; No. 30-49.

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