WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 1609-1622. Research Article ISSN 2277– 7105

CHEMICAL INVESTIGATION OF FATTY ACID, PHENOLIC CONTENTIN ARACHIS HYPOGAEA, ANACARDIUM OCCIDENTALE, PRUNUS DULCIS, PRUNUS ARMENIACA AND COMPARISON OF THEIR ANTIBACTERIAL ACTIVITY WITH AMOXICILLIN

***Agrawal Shelly, Malviya Shikha and Shukla Rudra Narayan**

Department of Applied Chemistry, Samrat Ashok Technological Institute, Vidisha (M.P.), 464001 India.

Article Received on
11 Sept 2015,

Revised on 03 Oct 2015,
Accepted on 25 Oct 2015,

***Correspondence for Author**
Agrawal Shelly
Department of Applied Chemistry, Samrat Ashok Technological Institute, Vidisha (M.P.), 464001 India.

ABSTRACT

Polyphenols are the widely distributed secondary metabolites in plants showing potent antioxidant properties against pathogenic bacteria, thus their identification and analysis has become a major area of research.The present study reveals the polyphenolic composition and antibacterial activity found in Arachis hypogaea, Anacardium occidentale, Prunus dulcis, Prunus armeniaca seeds. The phytochemical analysis of methanolic extract shows the presence of alkaloids, glycosides, tannins, phenolic group, flavonoids sterols. Quantitative analysis of vitamins shows that being rich in vitamin B complex, have high nutritional value. The total phenolic content determined by colorimeter reveals that P.K, C.K, A.K., Apr .S. Contain 59 mg/gm, 24 mg/gm, 22 mg/gm, 82 mg/gm GAE respectively whereas individual compounds detected by GC-MS technique shows the presence of d-Glycero-d-galacto-heptose, α-D-Galactopyranose, 6-O-(trimethylsilyl)-, cyclic 1,2:3,4-bis (methylboronate), Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 6-Octadecenoic acid, methyl ester, Oleic Acid ,13-Docosenamide, (Z)- in Arachis hypogeae, Cyclodecasiloxane, eicosamethyl-, Hexadecanoic acid, methyl ester, Estra-1,3,5(10)-trien-17β-ol, Cyclodecasiloxane, eicosamethyl-, 9-Octadecenamide, (Z)-,2,3-Dimethoxy-5-methyl-6-dekaisoprenyl-chinon, Cyclodecasiloxane, eicosamethyl inAnacardium occidentale, 4H-1,2,4-Triazole-3-thiol, 4-methyl-5-[5-(1-pentynyl)-3-pyridyl]-, Pentadecanoic acid, 14-methyl-, methyl ester, 10-Octadecenoic acid, methyl ester, 9-Octadecenamide, (Z)- in

Prunus dulcis, Cyclodecasiloxane, eicosamethyl-**,** Pentadecanoic acid, 14-methyl-, methyl ester**,** Dibutyl phthalate**,** 1-Monolinoleoylglycerol trimethylsilyl ether**,** 9-Octadecenamide, (Z)-**,**7-(4-Methoxy-phenyl)-cyclohepta-1,3,5-triene**,** Octasiloxane, 1,1,3,3,5, 5,7,7,9,9, 11,11, 13,13,15,15-**,** hexadecamethyl- in Prunus armeniaca respectively. The antimicrobial activity of methanolic extract against gram positive bacteria when compared with amoxicillin reveals that they exhibit good antibacterial property and thus can be used for therapeutic purpose in future.

KEYWORDS: Arachis hypogaea, Anacardium occidentale, Prunus dulcis, Prunus armeniaca, methanolic extract, vitamin analysis, total polyphenolic content, antibacterial property, amoxicillin GC-MS, UV Spectrophotometer.

INTRODUCTION

Nuts are an important nutritious food known because of their high fiber, mineral and vitamin content. Arachis hypogaea, Anacardium occidentale, Prunus dulcis, Prunus armeniaca, are some of the important nuts being used from ancient times. Arachis hypogeae grown in almost all tropical and subtropical countries are among the major oil seed crops grown in India. The seeds are rich in oleic acid, linoleic acid, palmitic acid, stearic acid and arachidic acid.[1] Anacrdium occidentale being popularly used as food source and in cuisine are rich in monosaturated fatty acid like oleic acid and palmitic acid.[2] On world wide basis Prunus duclis are one of the most popular tree nuts and rank number one in production. The edible portion of tree which is almond seed serves as rich source of nutrients consisting of of monosaturated fatty acid such as oleic acid, linoleic acid in abundance.[3] However Prunus Ducilis mostly grown in Central Asia and around mediterraneam consist of monosaturated fatty acids, polysaturated fatty acids and saturated fatty acid as well. Some common fatty acid found are oleic acid, linoleic acid etc.[4] Apart from their nutritional value these nuts are also an excellent source of polyphenols.[5-8] Polyphenols are natural compounds occurring in plants, however they are also present in foods such as fruits, vegetables, cereals etc.Chemically they are class of compounds consisting of hydroxyl group (OH) bond directly to aromatic ring. Among polyphenols phenolic compounds are classified as simple polyphenols based on the number of phenol units in the molecule.[9] On the basis of their chemical structure and complexity they are classified into two major groups flavonoids and non-flavonoids.[10] At present polyphenols are considered to be an ideal chemotherapeutic agents due to its non toxic properties.[11] Continuous research work has shown that

consumption of polyphenolic rich compound offers protection against development of cancers, and cardiovascular diseases, diabetes osteoporosis and neurodegenerative diseases.[12] Various researches conducted has shown that plant polyphenolics can be used as an antimicrobial drugs.[13] It has been found that polyphenolic fraction present in the skin covering the kernels of several nuts mostly composed of non flavonoids tannin structures (ellagitannins), flavan-3-ols and proanthocyanidin shows selective antimicrobial activities against gut pathogenic bacteria.[14] This antimicrobial and anti carcinogenic activities can be attributed to the antioxidant activities found in fruits due to polyphenols.[15] Thus polyphenols are considered to be the most bioactive compounds among all phytochemicals and are regarded as xenobiotics by the body.[16] Since the effects of polyphenols depend on their bioavailability and amount of consumption.[17] At present amoxicillin is one among the popular antibiotic drug being used.

The main aim of this research is to analyze the polyphenolic composition of Arachis hypogaea, Anacardium occidentale, Prunus dulcis, Prunus armeniaca, and determination of its antibacterial activity efficiency in comparison to synthetic drug amoxicillin.

MATERIALS AND METHODS

Collection of samples

The samples of Arachis hypogaeakernel (P.K) Anacardium occidentale kernel (C.K), Prunus dulcis kernel (A.K), and Prunus armeniaca seeds (Apr .S) were collected from local market of Vidisha.

Preparation of samples

The samples were dried at 100^0 C. in an oven for 1 hour. The samples were then grounded usingblender. The powdered sample was then distributed into 350g air tight plastic bags. for determination of physico chemical and phytochemical analysis.

Preparation of Crude Extracts

300 ml of methanol were taken into sterilized round bottom flasks of 500 ml capacity then added 200 gm of each dried powdered sample. It was kept undisturbed for 72 hours. Later the extracts were filtered through Whatman No.1 filter paper. The supernatants were collected separately, labeled and used for the screening of various phytochemicals.[18]

Extraction of polyphenolic content

10 g of each powdered sample were mixed with 150 ml of methanol and placed on water bath at 40 °C for24 h. The extracts were filtered and concentrated by rotary evaporator. The crude extract was freeze for 2-8^{0}C. The crude freeze (extract was kept in a desiccators filled with silica desiccant until further analysis).[19]

Physiochemical analysis

The moisture, ash and crude fiber content was determined by (AOAC 1990) method [20]. The acid soluble and insoluble was determined as per IP 2010 [21].Results are shown in table 2.The mineral calcium and magnesium was determined bycomplexometric titration using EDTA whereas iron and phosphate by colorimetric analysis [22].

Phytochemical identification:

The Phytochemical identification of methanolic extract of each sample was performed as per the method given in Rajinikanth Marka et,al 2013.The various test performed are given in table 1.

Table.1: Phytochemical identification methods

s.no	Name of phytochemical test	Method
1	Alkaloids	Dragendorff's test
2	Glycosides	Molish test
3	Tannins	Ferric chloride test
4	Phenols	Ferric chloride test
5	Flavonoids	Alkaline reagent test
6	Sterols	Salkowski test
7	Lignin	Furfuraldehyde test
8	Quinones	Alcoholic KOH test
9	Saponin	Foam test
10	Fats and oils	Stain test

Vitamin Analysis

Vitamin B_6, folates were determined by U.V spectrophotometric analysis.[23] while vitamin B_1, Niacin by perchlorictitration.[24]

Fatty acid composition by GC MS

The GC-MS profiling of extract was done using **Agilent 7890A GC coupled with 5975C MS system** as per methodGeneral 1 HP5 80 DEG.M.[25]

Total phenolic content analysis

Total phenolic content of the extracts was determined using Folin–Ciocalteu reagent as described by Spanos and Wrolstad with some modifications.[26]

Assay of amoxicillin

The assay determination of amoxicillin was performed as per Indian pharmacopeia 2007.[27]

Antibacterial activity

The fractions of methanolic extract were individually tested against bacteria. Using Nutrient agar medium as culturing media for strain. Antibacterial activity was revealed by growth inhibition in the test strain, observed in solid medium. In the present study we used Agar diffusion disc-variant method described by Cleidson Valgas et al.2007.[28]

RESULTS AND DISCUSSIONS

Physiochemical analysis

The proximate composition of P.K, C.K, A.K and Apr .S are shown in table 1.The results showed that A.K. contain highest moisture content 6.35 % whereas P.K contain least 5.1%.The crude fibre determination showed that P.K had highest crude fibre contentwith 4.3% while C.K. lowest with 1.4%However the ash determination showed that A.K. contain lowest ash content 3.8% whereas P.K maximum 4.2%.The mineral composition results of each samples shown in Table 3 reveals that they are rich in Ca, Mg, Fe and P and thus are helpful in treating many diseases.

Table.2: Proximate composition of samples

Parameters	P.K	C.K	A.K	Apr .S
Moisture	5.1%	5.8%	6.35%	5.8%
Crude fibre	4.3%	1.4%	3.7%	3.2%
Ash	4.2%	4.8%	3.8%	4%
Water soluble ash	50%	60%	60%	50%
Acid Insoluble ash	20%	34%	10%	12%

Table3. Mineral composition of samples (100 gm)

Minerals	P.K	C.K	A.K	Apr .S
Calcium	156.1 mg	298.8 mg	273.6 mg	39.04 mg
Magnesium	96.0 mg	64.0 mg	256 mg	64 mg
Iron	4.5 mg	6.8 mg	8.6 mg	3.8 mg
Phosphorous	81.6mg	417.6mg	393mg	73.6mg

PHYTOCHEMICAL RESULTS

The results phytochemical identification shown in Table 4 showed that methanolic extract of P.K, C.K, A.K and Apr .S contain alkaloids, glycosides, tannins, phenols, flavonoids, steroids, lignin, saponins, oils and fats.

Table 4: Identification of Phytochemicals in methanolic extract of samples

Phytochemical test	P.K.	A.K	C.K.	Apr.S
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Flavanoids	+	+	+	+
Steroids	+	+	+	+
Lignin	+	+	+	+
Quinone	-	-	-	-
Saponins	+	+	+	+
Oils and fats test	+	+	+	+

Vitamin analysis

The vitamin analysis of P.K, C.K, A.K and Apr.S showed that they are rich in vitamin and thus play an important role in treating a number of diseases. The results shown in Table 5 revealed that P.K, C.K., and A.K, consist largest proportion of Niacin with 12.554 mg/100 gm, 2.58 mg/100gm, 3.385 mg/100gm respectively whereas the Apr.S had Vitamin B1 10.270 mg/100 gm in abundance. However the further analysis shows the presence of folates, with 50 mg/100 gm in P.K., A.K. andVitamin B6 with 0.348 mg/100 gm respectively.

Table 5: Vitamin analysis in samples per 100 gm

VITAMIN	P.K.	A.K	C.K.	Apr.K
B_1	0.6721mg	0.230mg	0.644mg	10.270 mg
B_6	0.348mg	-	-	0.007 mg
NIACIN	12.554mg	2.58mg	3.385mg	-
FOLATES	0.050mg	0.050 mg	-	-

GC-MS RESULTS

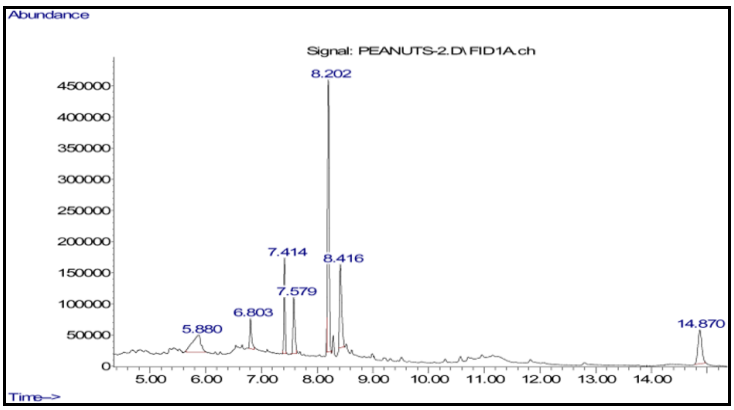


Fig 2: GC-MS Profiling of peanuts kernel extract

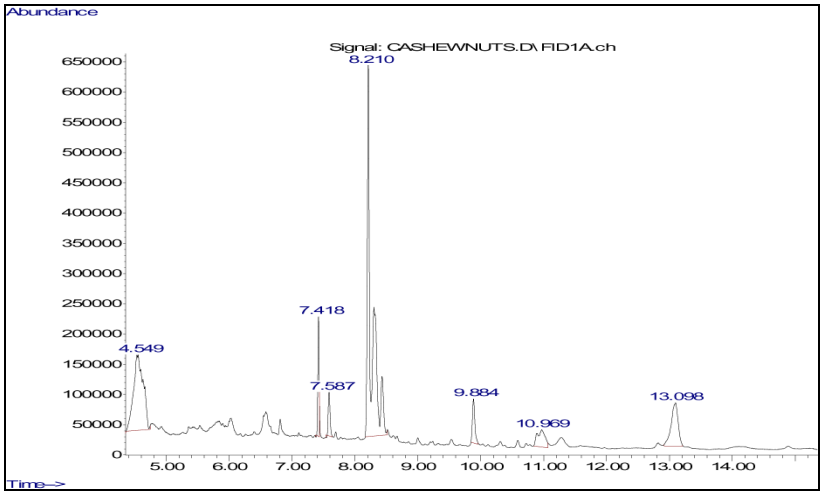


Fig 3: GC-MS profiling of cashew nut kernel extract

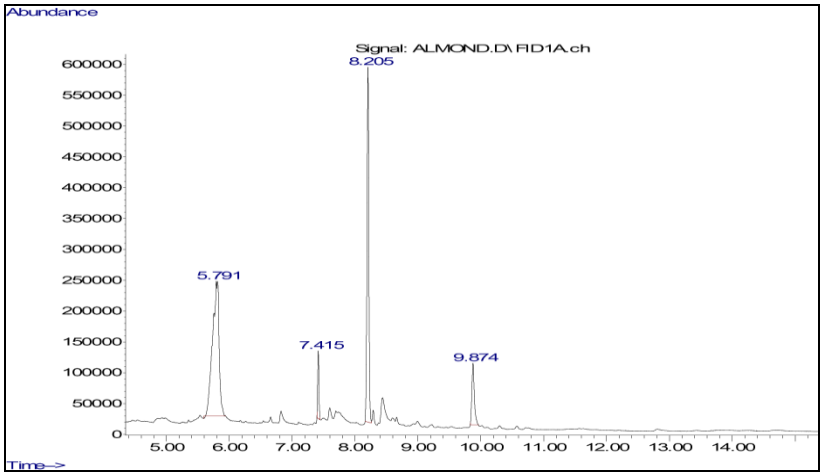


Fig 4: GC-MS Profiling of Almond kernel extract

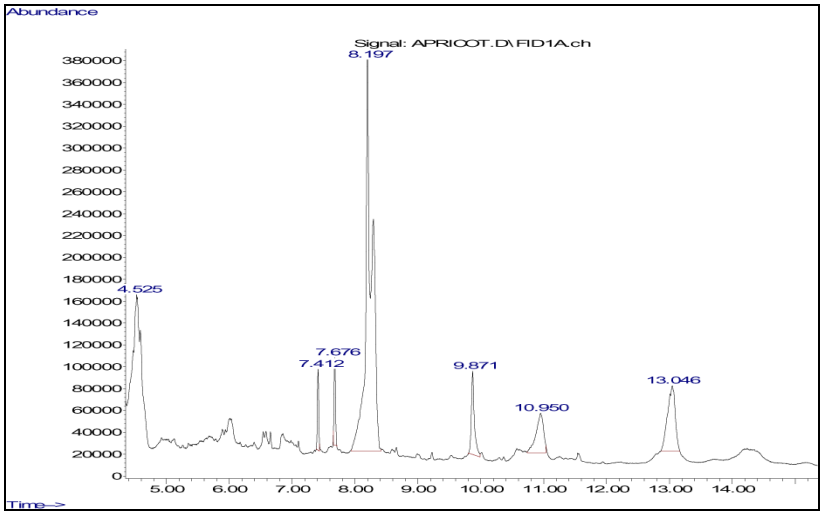
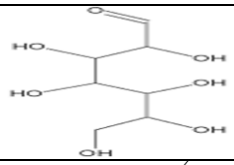
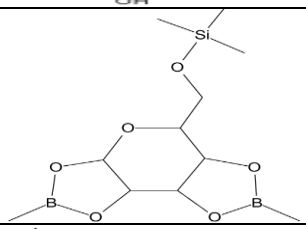
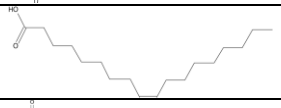
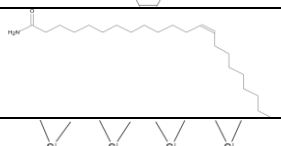
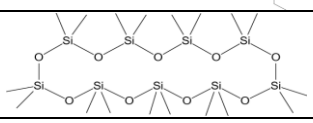
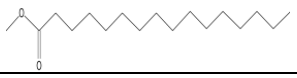
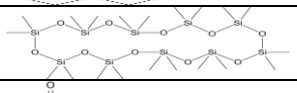
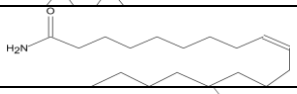
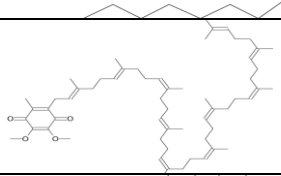
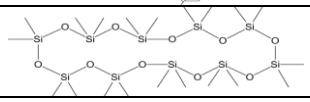
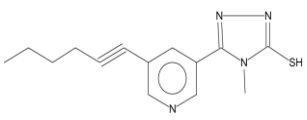
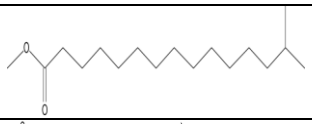
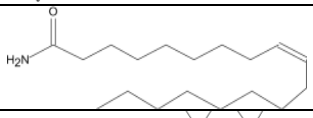
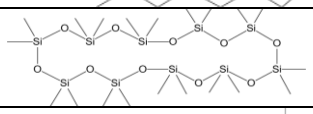
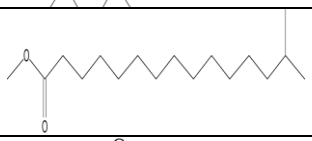
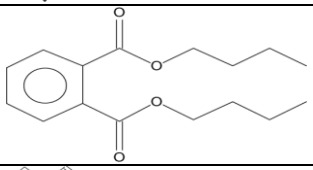
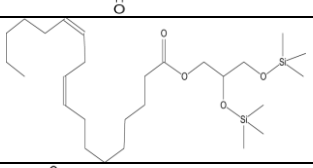
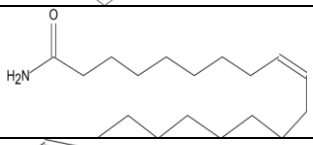


Fig 8: GC-MS profiling of Apricot seeds extract

Table 6: GC-MS profiling of Methanolic extract of each sample

S name	RT	Compound	Formula	Structure	MW	%
P.K	5.880	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$		210	12.598
	6.803	α-D-Galactopyranose, 6-O-(trimethylsilyl)-, cyclic 1,2:3,4-bis(methylboronate)	$C_{11}H_{22}B_2O_6Si$		300	4.730
	7.414	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		270	9.692
	7.579	Palmitic acid	$C_{16}H_{32}O_2$		256	9.004
	8.202	6-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		296	37.908
	8.416	Oleic Acid	$C_{18}H_{34}O_2$		282	15.115
	14.870	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$		337	10.953
C.K	4.549	-Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$		740	25.365
	7.418	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		270	5.062
	7.587	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$		256	2.791
	8.210	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$		740	48.200
	9.884	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$		281	3.705
	10.969	2,3-Dimethoxy-5-methyl-6-dekaisoprenyl-chinon	$C_{59}H_{90}O_4$		862	4.580
	13.098	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$		740	10.296
	5.791	4H-1,2,4-Triazole-3-thiol, 4-methyl-5-[5-(1-pentynyl)-3-pyridyl]-	$C_{14}H_{16}N_4S$		272	50.494

A.K	7.415	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$		270	4.766
	8.205	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		296	36.162
	9.874	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$		281	8.578
Apr.S	4.525	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$		740	16.584
	7.412	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$		270	2.462
	7.676	Dibutyl phthalate	$C_{16}H_{22}O_4$		278	2.879
	8.197	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$		498	54.725
	9.871	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$		281	5.525
	10.950	7-(4-Methoxy-phenyl)-cyclohepta-1,3,5-triene	$C_{14}H_{14}O$		198	6.628
	13.046	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$		578	11.196

Fatty acid composition

TheGC-MS analysis of each sample showed that they are rich in fatty acids and thus can be used as an oilseed crop. Fig 1-4 represents the graphical presentation of analysis whereas the components obtained are shown in Table 6. The results showed that 6-Octadecenoic acid, methyl ester accounts for highest 37.908 % in peanuts followed by Oleic Acid 15.115%, d-Glycero-d-galacto-heptose 12.598%, 13-Docosenamide, (Z)-10.953 % in abundance. However Cyclodecasiloxane, eicosamethyl accounts for maximum 48.200 % in Ancardium occidentale followed by Cyclononasiloxane, octadecamethyl-25.365 %, Cyclodecasiloxane, eicosamethyl- 10.296% abundance. The other sample Prunus duclis consist of 50.594 % of 4H-1,2,4-Triazole-3-thiol, 4-methyl-5-[5-(1-pentynyl)-3 pyridyl] in maximum and Prunus armeniaca consist of 50 % 1-Monolinoleoylglycerol trimethylsilyl ether in abundance. Thus

all results revealed that Arachis hypogeae, Anacardium occidentale, Prunus duclis, Prunus armeniaca are rich in monosaturated fatty acids and thus used as an important oilseed crops

Total phenolic content

The results of total phenolic content found in methanolic extract of P.K, C.K, A.K and Apr .S are shown in Figure 1. The results showed that P.K, C.K, A.K and Apr .S contain 24 mg/gm , 59 mg/gm , 22 mg/gm and 82 mg/gm of TPC respectively.

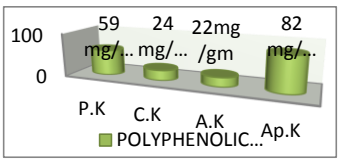


Fig .1: Total polyphenolic content found in methanolic extract of samples

Assay of Amoxicillin

The fig 6 ,7 represents the absorption graph observed in UV spectrophotometer.The analysis of synthetic drug by U.V spectrophotometer as per parameters showed that the assay purity of amoxicillin was 91.4% and thus was found suitable.

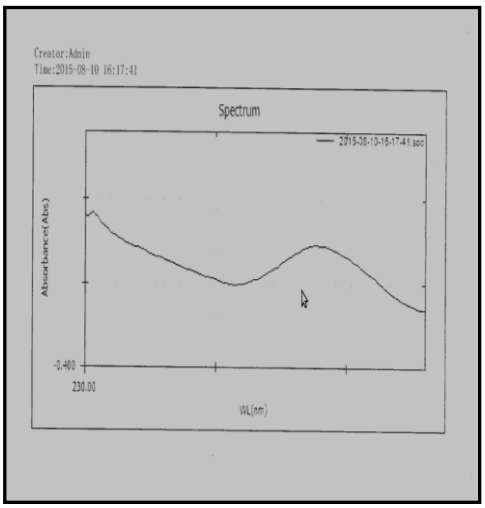


Fig. 6 peak abs.:0.5350 at 320 nm (Standard)

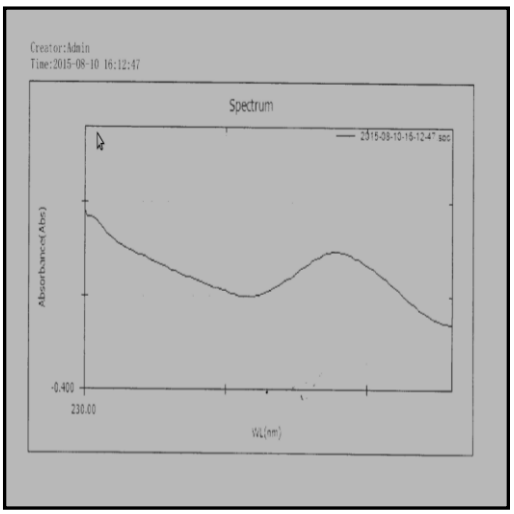


Fig. 7 peak abs.:0.5270 at 320 nm (Sample)

Antibacterial activity

The microbiological assay of each extract revealed that they exhibit good antibacterial activity. However the comparison among extract and amoxicillin showed that P.K have comparable zone of inhibition with that of amoxicillin in comparison to other

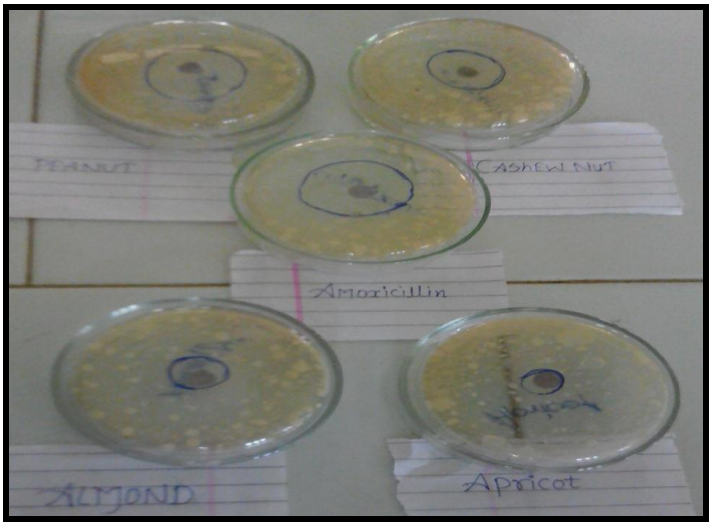


Fig .8: zone of inhibition of methanolic extract and amoxicillin against bacterial strain

Table 7: Antibacterial activity of methanolic extract and amoxicillin zone of inhibition

s.no	Name of sample	Zone of inhibition
1.	P.K	17mm
2.	C.K	13mm
3.	A.K	9mm
4.	Ap.k	7mm
5.	Amoxicillin	17.5mm

CONCLUSION

The GC-MS results shows that nuts are rich in oleic acid, palmitic acid and linoleic acid and thus are being used as an oil seed crop. However the analysis of total phenolic content reveals that can also be used as antioxidants. The comparison of antibacterial activity of all nuts with that of amoxicillin showed that P.K. exhibited maximum antibacterial activity followed by C.K, A.K., Apr.S in comparison to amoxicillin. Thus it can be concluded that these nuts being rich in nutritional value also shows antibacterial property due to presence of phenolic content in them and hence can be used as an antibiotic as well. However the further analysis on it can lead to the development of new chemotherapeutic agent which may be helpful in treating chronic diseases like Cancer.

ACKNOWLEDGEMENT

We are very grateful to Dr. J.S. Chauhan I/C Director S.A.T.I., Vidisha, Mr.J.P.Saxena controller, Food and Drugs Lab. Bhopal, CIF Mass facility, IISER Bhopal,for helping tocarry out our analyticalwork.

REFRENCES

1. Khaled Sebei, Asma Gnouma, Wahid Herchi, Faouzi Sakouhi, Sadok Boukhchina Lipids, proteins, phenolic composition, antioxidant and antibacterial activities of seeds of peanuts (Arachis hypogaea l) cultivated in Tunisia Biol. Res, 2013: 46: 3. Santiago ISSN 0716-976.
2. "Basic Report: 12085, Nuts, cashew nuts, dry roasted, without salt added". Agricultural Research Service –United States Department of Agriculture United States Department of Agriculture. Archived from the original on, June 2014; 06-18.
3. Hari Jagannadha Rao, Lakshmi Therapeutic applications of almonds Prunus amygdalulus: A reviewJournal of Clinical and Diagnostic Research. February, 2012; 6(1): 130-135.
4. Allie Hanson (2013). The Astounding Acai Berry. Published by Department of Nutrition and Dietetics, Concordia College, Moorhead, MN. Retrieved May 23, 2013.
5. Ali Esmail Al-Snafi ,Chemical Constituents and Pharmacological Activities of Arachis hypogaea International Journal for Pharmaceutical Research Scholars (IJPRS), 2014; 3: 1. ISSN No: 2277–7873
6. Rune Blomhoff, Monica H. Carlsen, Lene Frost Andersen and David R. Jacobs "Health benefits of nuts: potential role of antioxidants". British Journal of Nutrition, November 2006; 96 (2): 52–60.
7. Ali Jahanban Esfahlan, Rashid Jamei , Rana Jahanban Esfahlan The importance of almond (Prunus amygdalus L.) and its by-products Food Chemistry, 2010; 120: 349–360
8. Federica Sotgia, Ubaldo E Martinez-Outschoorn, and Michael P Lisanti (May 2011). Mitochondrial oxidative stress drives tumor progression and metastasis: should we use antioxidants as a key component of cancer treatment and prevention? Published in BMC Medicine. Retrieved May 23, 2013.
9. Robbins, Rebecca J. "Phenolic Acids in Foods: An Overview of Analytical Methodology". Journal of Agricultural and Food Chemistry, 2003; 51: 2866–2887.
10. Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G. and Crozier, A. Dietary (poly) phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects against Chronic Diseases. Antioxidants & Redox Signaling, 2012; 18: 1-73.
11. Rahul K. Lall, Deeba N. Syed, Vaqar M. Adhami, Mohammad Imran Khan and Dietary Polyphenols in Prevention and Treatment of Prostate Cancer Int. J. Mol. Sci, 2015; 16: 3350-3376. ISSN 1422-0067.

12. Kanti Bhooshan Pandey and Syed Ibrahim RizviPlant polyphenols as dietary antioxidants in human health and disease Oxid Med Cell Longev, Nov-Dec 2009; 2(5): 270–278. PMCID: PMC2835915.
13. Ferrazzano, G. F., Amato, I., Ingenito, A.,Zarrelli, A., Pinto, G. & Pollio, A. Plant polyphenols and their anti-carcinogenic properties:a review Molecules, 2011; 16: 1486–1507.
14. Fernando Cardona, Cristina Andrés-Lacueva, Sara Tulipani, Francisco J. Tinahones,María Isabel Queipo-Ortuno, Benefits of polyphenols on gut microbiota and implications in human health Journal of Nutritional Biochemistry, 2013; 24: 1415-1422.
15. Mandalari G, Faulks RM, Bisignano C, Waldron KW, Narbad A, Wickham MS. In vitro evaluation of the prebiotic properties of almond skins (Amygdalus communis L.). FEMS Microbiol Lett, 2010; 304(2): 116–22.
16. Arjan Scheepens.,Kee Tan • James W. Paxton Improving the oral bioavailability of beneficial polyphenols through designed synergies Genes Nutr, 2010; 5: 75–87. DOI 10.1007/s12263-009-0148-z.
17. Claudine Manach, Augustin Scalbert, Christine Morand, Christian Remesy and Liliana Jimenez, Polyphenols: food sources and bioavailabilityAmerican Journal of Clinical nutrition, May 2004; 79: 5 727-747.
18. Rajinikanth Marka, Samatha Talari, Srinivas Penchala, Shyamsundarachary Rudroju, Rama Swamy Nanna Preliminary Phytochemical Analysis of Leaf, Stem, Root and Seed Extracts of Arachis hypogaea L Int. J. Pharm. Sci. Rev. Res., 22 May – Jun 2013; 20(1): 134-139.
19. VV Kiat 1, Wei Kit Siang 1, P Madhavan2, JH Chin 1, M Ahmad 3, GA Akowuah1 FT-IR Profile and Antiradical Activity of Dehulled Kernels of Apricot, Almond and Pumpkin. Research Jou Sciences rnal of Pharmaceutical, Biological and Chemical Sciences ISSN: 0975-8585
20. AOAC. Official methods of Analysis. 15th Edn. Wahsington D.C., 1990; 375-379.
21. 1ndian Pharmacopeia. 2007; 1(2): 3-16.
22. J.Mendham., R. C. Denney., J.D.Barnes., M. J. K .Thomas., Vogel's : Text Book of Quantitative Chemical Analysis.6th edition Published by Pearson Education (Singapore) Pvt.Ltd, 2004.
23. Brassica Pharmaceuticals & Chemicals T-68, M.I.D.C. Tarapur, Boisar.
24. Method of analysis of vitamins, provitamins and chemically well defined substances having a similar biological effect E033-Fefana.xp 22/12/05

25. CIF-Mass Facility "Indian Institute of Science Education and Research" Bhopal (M.P.)
26. Spanos GA, Wrolstad RE. J Agric Food Chem, 1990; 38: 1565-1571.
27. Indian pharmacopeia, 2007.
28. Cleidson Valgas et al. screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology, 2007; 38: 369-380. ISSN 1517-8382.