Study of Antimicrobial Activities and Optimization of Plant Extract Preparation of *Mangifera indica*; The King of Fruits

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Abstract - The cold water, hot water, methanol suspension, Methanol column & methanol soxhelt extract of Mangifera indica peel powder was subjected to antimicrobial bioassay. Antibacterial activity was assayed against three pathogens namely Pseudomonas aeruginosa.E.coli and S.aureus for aqueous and methanolic extract of dry powder of M.indica peel. Among all hot water extract showed Maximum activity against S.aureus with 2.20 cm of zone of inhibition. Methanol suspension extract was best giving large zone of inhibitation against all the pathogens. The MIC of cold water was 4.28 mg/ml against Pseudomonas aeruginosa followed by methanol column was 10.71 mg/ml against E.Coli and S.aureus.

Keywords - *Mangifera indica*, Antimicrobial

Introduction

Mangifera indica (MI) also known as mango, aam, has been an important tree in the Ayurvedic and indigenous medical systems for over 4,000 years. Mangoes belong to genus Mangifera which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae. According to Ayurveda, varied medicinal properties are attributed to different parts of mango tree. Mango is one of the most popular of all tropical fruits. Mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone, has strong antioxidant, anti lipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities (**Shankarnarayanan et. al, 1979**). The genus Mangifera originates in tropical Asia with the greatest number of species found in Borneo, Java, Sumatra and the Malay Peninsula. The most-cultivated Mangifera species, M. indica (mango) has its origins in India and Myanmar. Chemical constituents of MI are always of an interest. The different chemical constituents of the plant are especially the polyphenolics, flavonoids, triterpenoids, Mangiferin a xanthone glycoside (major bio-active constituent) isomangiferin, tannins & gallic acid derivatives. The bark is reported to contain protocatechic acid, catechin, mangiferin, alanine, glycine, γ-aminobutyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3β,26diol, 3-ketodammar-24 (E)-en-20S,26-diol, C-24 epimers of cycloart-25 en 3β, 24, 27-triol and cycloartan-3β, 24, 27-triol.(Chen et. al, 2004). Indicoside A and B, manghopanal, mangoleanone, friedelin, cycloartan-3β-30-diol and derivatives, mangsterol, manglupenone, mangocoumarin, n-tetacosane, n-heneicosane, n-triacontane and mangiferolic acid methyl ester and others isolated from stem bark of MI. Mangostin, 29-hydroxy mangiferonic acid and mangiferin have been isolated from the stem bark together with common flavonoids. The flower yielded alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propyl gallate, n-pentyl gallate, n-octyl gallate, 4-phenyl gallate, 6-phenyl-n-hexyl gallate and dihydrogallic acid. Root of mango contains the chromones, 3-hydroxy-2-(4'-methylbenzoyl)-chromone and 3-methoxy-2-(4'-methyl benzoyl)-chromone. The leaf and flower yield an essential oil containing humulene, elemene, ocimene, linalool, nerol and many others. The fruit pulp contains vitamins A and C,β-carotene and xanthophylls. An unusual fatty acid, cis-9, cis-15-octadecadienoic acid was isolated

from the pulp lipids of mango. Phenolic antioxidants, free sugars and polyols isolated and analyzed from Mango (MI) stem bark. Quantitative analysis of the compounds has been performed by HPLC and mangiferin was found to be the predominant component. The natural C-glucoside xanthone mangiferin [2-C-β-Dgluco-pyranosyl-1, 3, 6, 7-tetrahydroxyxanthone] C19 H 18 O 11 ; Mw, 422.35; anhydrous has been reported in various parts of MI leaves, fruits, stem bark, heartwood and roots (Andreas et. al., 2000). The presence of a phenolic compound from leaves of MI was named as homomangifirin. The extract of mango showed a powerful scavenging activity of hydroxy radicals and acted as a chelator of iron. It also showed a significant inhibitory effect on the peroxidation of rat brain phospholipid and prevented DNA damage caused by bleomycin or copper-phenenthroline system (Maxwell, 1997). In vitro, antioxidant and free radical scavenging properties of a stem bark aqueous extract of mango tree (MI), whose formulations are used in Cuba as food supplements under the brand name of Vimang. The potential anti-diarrhoeal activity of methanolic (MMI) and aqueous (AMI) extracts of seeds of MI has been evaluated in experimental diarrhoea, induced by castor oil and magnesium sulphate in mice. The results illustrate that the extracts of MI have significant anti-diarrheal activity and part of the activity of MMI may be attributed to its effect on intestinal transit.

Material and Methods

Peels of Mangifera indica were purchased from the local market of Gomti Nagar, Lucknow. The mango peels were dried in the incubator and grinded in electronic blender to fine powder and were subjected to bioactive compounds extraction. The extract was obtained with help of various methods and solvents such as hot water extract, cold water extract, organic suspension extract, organic column extract and organic Soxhlet extract. All the extracts were dried up to crystal form and were dissolved in distilled water at concentration of 500mg/ml.

Bacterial cultures of Staphylococcus aureus (Gram positive), Pseudomonas aeruginosa (Gram negative) and Escherichia coli (Gram negative) were obtained from IMTECH, Chandigarh, and were sub-cultured on to petri plate containing nutrient agar media. Single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37 °C in shaker incubator at 140 rpm for 14 hrs. Bacterial cells were recovered by centrifugation and were suspended in sterile distilled water, concentration of pathogens was optimized by maintaining OD to 0.1 at 600 nm before use.

The antimicrobial activity of Mangifera indica peel powder was determined by agar well diffusion method against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. 10.0 ml nutrient agar media was poured in a sterile petri dish, 100 µl of test organisms were spread on the surface of media, wells were prepared with help of sterile borer, which were aseptically filled by 30µl plant extracts with positive (Tetracycline; 50µg/ml) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 °C for 14 hrs. The diameters of zones of inhibition were measured.

Active extracts obtained by agar well diffusion assay were further subjected to determine the Minimum Inhibitory Concentration (MIC) required for the bacterio-static effects by standard micro-dilution agar double layer methodology. This is carried out by double agar gradient plate method. Nutrient agar (5.0 ml) was poured into sterilized petri dishes, leaving the plate in slanted position. After setting the media, another 5.0 ml of nutrient agar (along with plant extract; 5.0 mg/ml) was added to the plates to make the level unity; thus the plate contained an increasing concentration of plant extract along the diameter of the plate. Now the 70µl of prepared inoculums of cultures were spread. Plates were incubated in upright position at 37 °C for 14 hrs. Concentration gradient along with the diameter was calculated for each mm. visible colonies were observed, distance was measured from top end and concentration of the compound was calculated as MIC.

sults

traction was carried out using hot water extraction, cold water extraction, Methanol column, thanol suspension and Methanol Soxhlet.

Extraction Method	Yield obtained (grams)	Color of the extract obtained
Hot water extraction	0.610	Dark yellow
Cold water extraction	1.138	Dark brown
Methanol suspension	0.610	Dark yellow
Methanol column	1.329	Blackish brown
Methanol Soxhlet	3.447	Dark brown

Table 1- Yield of each extract of Mangifera indica peel powder

ntibacterial activities of all the extracts were assayed against Pseudomonas aeruginosa, Escherichia coli nd Staphylococcus aureus by agar well diffusion method.

Organisms	*P. aeruginosa*	*E. coli*	*S. aureus*
Tetracycline(avg)	1.88 cm	2.20 cm	2.50 cm
Extracts (500mg/ml)			
Hot water extraction	1.40 cm	1.30 cm	2.20 cm
Cold water extraction	1.60 cm	1.80 cm	1.50 cm
Methanol suspension	2.00 cm	2.10 cm	2.00 cm
Methanol column	1.80 cm	1.70 cm	1.90 cm
Methanol Soxhlet	1.70 cm	1.60 cm	1.50 cm
Distilled water	0.00 cm	0.00 cm	0.00 cm

Table 2- Antibiogram of aqueous and methanolic extract against P. aeruginosa, E. coli and S. aureus.

Extracts found to have inhibitory effects were tested for determination of MIC by agar double layer method against susceptible bacterial species.

Test organisms/ Extracts(500mg/ml)	Minimum Inhibitory Concentration (in mg/ml)		
	P. aeruginosa	*E. coli*	*S. aureus*
Hot water extraction	16.71	15.00	17.57
Cold water extraction	4.28	12.85	12.00
Methanol column	17.14	10.71	10.71
Methanol Soxhlet	15.00	25.00	19.28

Table 3- MIC (in mg/ml) of Mangifera indica peel extract (aqueous and methanolic) against P. aeruginosa, E. coli and S. aureus.

Discussion

Results of present investigation reveal the antibacterial nature of methanolic and aqueous extract of peel of Mangifera indica. The peel of Mangifera indica was extracted in the dry powder form using aqueous and methanol solvent. Data of Table 1 demonstrates that maximum yield was obtained by using methanol Soxhlet extraction method.

Antibacterial activity was assayed against all three pathogens namely, P. aeruginosa, E. coli and S. aureus for aqueous and organic extracts of M. indica peel and among all, hot water extract proved maximum activity against S. aureus with 2.20cm of zone of inhibition. While methanol suspension extract was best extraction procedure by giving large zone of inhibitions against all the pathogens selected in the study (Table 2, fig 3, fig 4, fig 5). The Minimum Inhibitory Concentration of hot water extract, cold water extract, methanol column and methanol soxhlet was carried out. Cold water recorded MIC of 4.28mg/ml against P. aeruginosa followed by methanol column with MIC of 10.71 mg/ml against E. coli and S. aureus.

Sterile distilled water which was used as negative control showed no zone of inhibition in any test organism. The efficacies of all extracts were less than that of the standard antibiotic tetracyclin Tetracycline showed average zone of inhibition 2.50cm against all the test organism.

References

Abubakar, M. E. 2009. Antibacterial efficacy o stem bark extracts of Mangifera indica agains some bacteria associated with respiratory trac infections. Sci. Res. & Essay 4 (10): 1031-1037

Ajila, C.M.; Leelavathi, K.; & Prasada Rao, U.J.S 2007. Improvement of dietary fiber content an antioxidant properties in soft dough biscuits wit the incorporation of mango peel powder. J. Cerea Sci 48(2): 319-326

Ashoush, I.S.& Gadallah, M.G.E. 2011. Utilization o Mango Peels and Seed Kernels Powders a Sourcesof Phytochemicals in Biscuit. World J. Dair & Food Sci. 6 (1): 35-42,

Carvalho, A.A.T.; Vanetti, M.C.D.; Mantovani , H.(2008. Bovicin HC5 reduces thermal resistance o Alicyclobacillus acidoterrestrisin acidic mang pulp. J. App. Microbiol. 104(6): 1685-1691

Chanda, S.; Baravalia, Y.; Kaneria, M. & Rakholiya K. 2010. Fruit and vegetable peels - strong natura source of antimicrobiolbics. Int. J. Antimicrobi Agents: 444 - 450.

Engels, C.; Knödler, M.; Zhao, YY.; Carle, R.; Gänzle MG.; Schieber, A. 2009. Antimicrobiolbial Activit

Gallotannins Isolated from Mango (Mangifera ndica L.) Kernels. J. Agric. Food Chem 57(17):7712-718

arrido, G.; González, D.; Lemus, Y.; García, D.; odeiro, L.; Quintero, G.; Delporte, C.; Núñez-ellés, AJ.; Delgado, R. 2004. In vivo and in vitro nti-inflammatory activity of Mangifera indica L. xtract (VIMANG). 50(2): 143-149

upta, Charu; Garg .P, Amar; & Uniyal, Ramesh. 008. Antibacterial activity of Amchur (Dried Pulp f Unripe Mangifera indica) extracts on some food orne bacteria. J. Pharm. Res. 1(1): 54-57

uliana, A. S.; Zeila, P. L.; Hélio, K.; Alba, R.; Monteiro, S. B.; Lourdes, C. d. S.; Wagner, V. & lélia, A. H. L. 2009. Molecules.14: 1098 - 1110

hammuang, S. & Sarnthima, R. 2011. Antioxidant nd antibacterial activities of selected varieties of hai mango seed extract. Afr. J. biomed. Res. 21(1): 7 - 42

Mahida, Y. & Mohan, J. S. S. 2007. Screening of lants and their potential antibac activity against taphylococcus and salmonella spc. Natural Prod. Radiance. 6(4): 301-305

Masibo, M & He, Q. 2009. In vitro antimicrobiolbial ctivity and the major polyphenol in leaf extract of Mangifera indica L. Malaysian J. Microbiol. 5(2): 73-80

Mirghani, E.S.; Yosuf, F.; kabbashi, N.A.; Vejayan, J. & Yosuf, Z.B.M. 2009. Antibacterial Activity of Mango Kernel Extracts. Journal of Applied Sciences, 9: 3013-3019.

Nikhal, S.; Mahajan, S.D. 2010. Evaluation of antibacterial and antioxidant activity of Mangifera indica (leaves). J. Pharm. Sci. & Res. 2(1): 45-47

Ravi, V.; Prabhu, M.; Subramanyam, D. 2001. Isolation of bacteriocin producing bacteria from mango pulp and its antimicrobiolbial activity. J. Microbiol. & Biotech. Res. 1(2): 54-63

Sairam, K.; Hemalatha, S.; Kumar, A.; Srinivasan, T.; Ganesh, J.; Shankar, M.; Venkataraman, S. 2003. Evaluation of anti-diarrhoeal activity in seed extracts ofMangifera indica. J. Ethanopharma. 84(1): 11-15

Sowmiya, S.; Soundarapandian, P.; & Rajan, S. 2009. Bioactive Studies of Mangifera indica aganist Bacteria Isolated from Urine Samples. Current Res. J. Bio.Sci. 1(3): 139-143

Susy Mary Souto de Oliveir; 1 Vivyanne S. Falcão-Silva; 2 José P.Siqueira-Junior; 2 Maria José de Carvalho Costa; 3 Margareth de Fátima F. de Melo Diniz. 2011. Modulation of drug resistance in Staphylococcus aureus by extract of mango (Mangifera indica) peel, Brazilian J. Pharmacognosy. 21(1): 190-193.

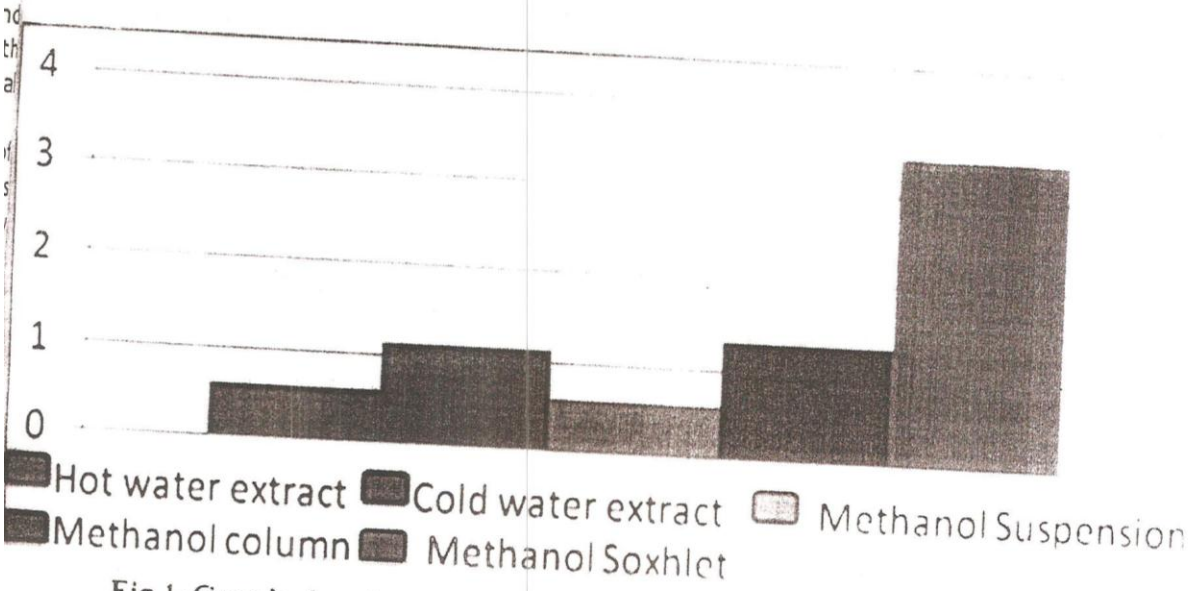

Fig.1- Graph showing yield of all the *Mangifera indica* peel extracts

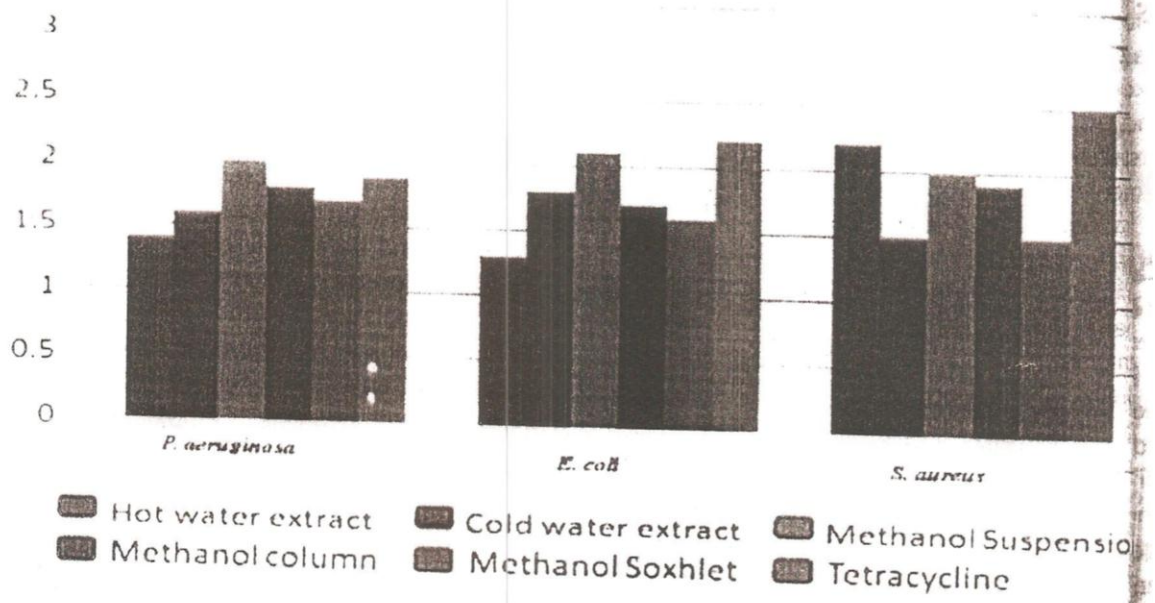

Fig.2- Graph showing comparative study of inhibition of all the extracts against test organism

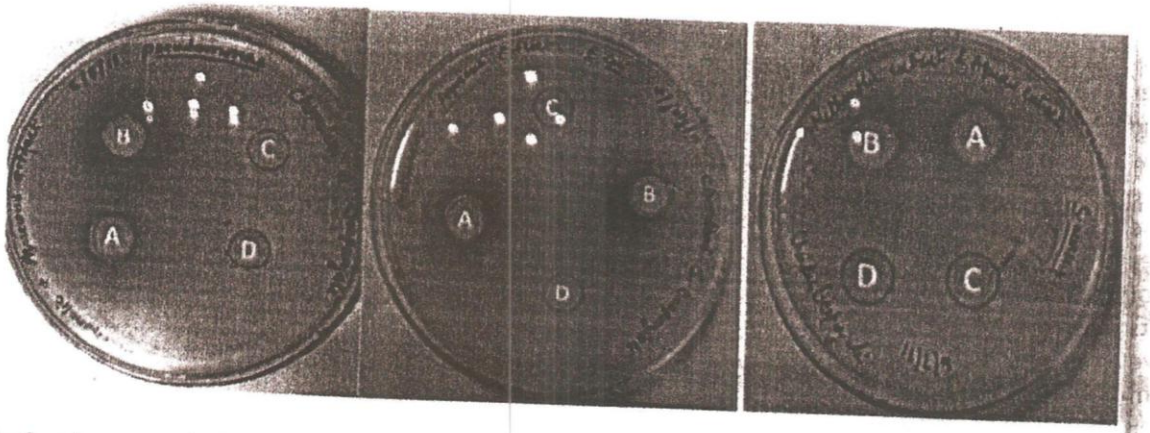

Fig 3- photograph showing antibiogram of the methanolic and aqueous extract of *Mangifer indica* peel against *P .aeruginosa, E. coli and S. aureus* respectively. (A-Methanol suspension. B-Cold water, C- Tetracycline, D- Distilled water)

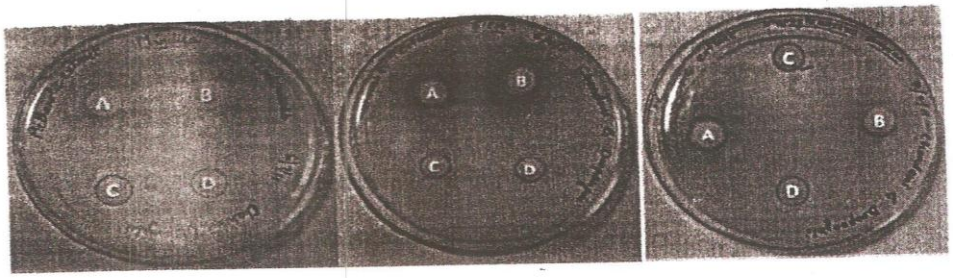

Fig 4- Photograph showing antibiogram of the methanolic extract of *Mangifera indica* peel against *P. aeruginosa, E .coli and S .aureus* respectively. (A-Methanol column. B-Methanol soxhlet. C- Tetracycline. D- Distilled water)

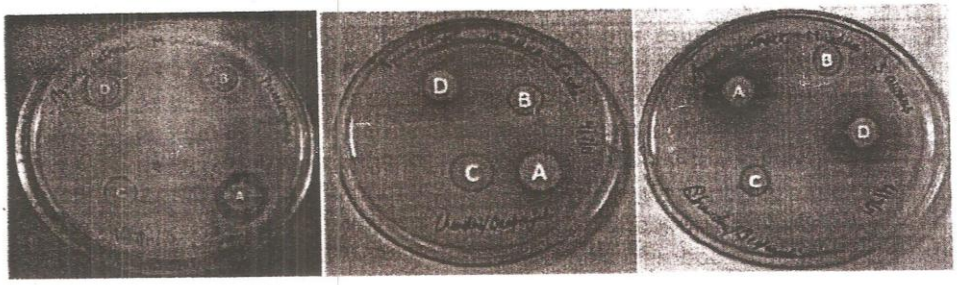

Fig 5- Photograph showing antibiogram of the Aqueous extract of *Mangifera indica* peel against *P. aeruginosa, E. coli and S. aureus* respectively. (A-Cold water extract. B-Tetracycline. C-Distilled water. D – Hot water extract)

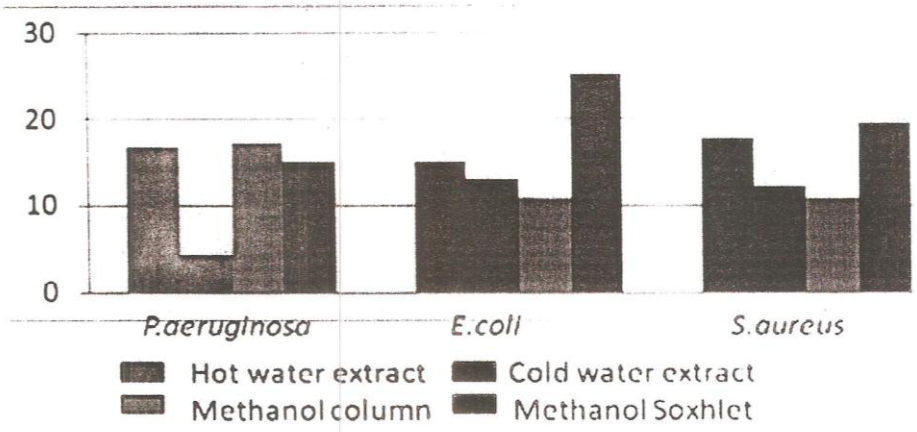

Fig6- Graph showing comparative study of MIC of hot water extract. Cold water extract. Methanol column and Methanol soxhlet against *P. aeruginosa, E. coli and S. aureus* respectively