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Review Article

PHARMACOLOGICAL POTENTIALS OF *CITRUS PARADISI* - AN OVERVIEW

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ABSTRACT

Grapefruit (*Citrus paradisi*) is an important member of *Citrus* genus from family Rutaceae. It has been used as a folk medicine in many countries as antibacterial, anti fungal, anti-inflammatory, antimicrobial, antioxidant, antiviral, astringent, and preservative. It has also been used for cancer prevention, cellular regeneration, lowering cholesterol, cleansing, detoxification, heart health maintenance, Lupus nephritis, rheumatoid arthritis and weight loss. This review is intended to highlight the pharmacological activities of this plant for various therapeutic uses so as to enable the researchers to view all the potentials at a single platform. This may help the scientists in designing new drugs with varied activities in a single formulation.

Key words: - *Citrus paradisi*, Grapefruit, Medicines, Rutaceae.

INTRODUCTION

Citrus paradisi is the one of the most important member of the genus citrus (Rutaceae). It is native to the island of Barbados. Grapefruit are also grown commercially in Spain, Morocco, Israel, Jordan, South Africa, Brazil, Mexico, Jamaica, and Asia [1]. Further varieties of Grapefruit were developed mainly in Florida and Texas, USA [2]. Today it's the second most important *citrus* worldwide and a key commercial crop in the U.S. states of Texas, Arizona, California and Florida. In Florida alone, more than 2.5 million tonnes of grapefruit are harvested annually. The U.S. now produces 60% of the world's grapefruit crop [3].*Citrus paradisi* is an important fruit in Pakistan and its production is increasing day by day due to its considerable medicinal importance. The most significant byproduct of this massive processing industry is the several essential oils, which are of consumer and economic importance. It is also used as ingredient of cosmetic, perfumes, soaps and detergents.

The approximate yield of essential oil from citrus paradise is 3.9% which contains open chain hydrocarbons, alcohols, aldehydes, ketones, esters and alpha-terpenoids [4]. As a relatively new food, the grapefruit has made great advances in the past 75 years. The sections are commonly used in fruit cups or fruit salads, in gelatins or puddings and tarts. In Australia, grapefruit is commercially processed as marmalade. It may also be made into jelly. The juice is marketed as a beverage fresh, canned, or dehydrated as powder, or concentrated and frozen. It can be made into excellent vinegar or carefully fermented as wine. Grapefruit peel is candied and is an important source of pectin for the preservation of other fruits. The peel oil, expressed or distilled, is commonly employed in soft-drink flavoring, after the removal of 50% of the monoterpenes. Naringin, extracted from the inner peel is used as a bitter in "tonic" beverages, bitter chocolate, ice cream and ices. Keeping in view its varied use, it is pertinent to explore medicinal importance of this plant. This compilation is an effort by authors to provide an opportunity to scientific fraternity to get all the information related to this plant at a single platform so that new formulations with multiple uses can be designed [5-6].

CHEMICAL CONSTITUENTS

Citrus peel is rich in flavanone glycosides and polymethoxyflavones. *Citrus paradisi* (grapefruit) the most interesting for isolation. The results show that the Star Ruby grapefruit stand out for their high contents of naringin. The presence of the polymethoxyflavones nobiletin, heptamethoxyflavone and tangeretin, could be ascertained in all the grapefruit varieties analyzed. Higher polymethoxyflavone levels were recorded in orange, with Valencia Late showing the greatest nobiletin, sinensetin and tangeretin contents and Navelate the highest heptamethoxyflavone levels. Naringin [the 7-β-neohesperidoside of narigenin (4', 5, 7-trihydroxiflavananone)] is found in citrus plants and is most abundant in *Citrus paradisi* species [7-8]. The hydro-distilled essential oil content from fresh-, ambient and oven-dried peels of *C. paradisi* ranged 0.20-0.40 g/100 g [9]. From the gas chromatography (GC) and GC/MS studied the essential oil shows the presence of 23 constituents out of which some constituents were identified as alpha-pinene (0.44%), beta-pinene (2.51%), limonene (81.6%), p-cymene (3.6%), linalyl acetate (5.20%), sabinene (1.02%), 4-terpineol (0.389%), alpha-terpineol (0.31%), alpha-thugene (0.28%), ctanol (0.26%), 1,8- cineol (0.42%), Geraniol (0.21%) and decanal (0.16%) [4]. Grapefruit pulp contains significant levels of vitamin C; potassium, folate, calcium, and iron. The pink and red varieties also contain beta-carotene and lycopene, antioxidants that the body can convert to vitamin A. Other protective plant chemicals found in grapefruits include phenolic acid, limonoids, terpenes, monoterpenes, D-glucaric acid and flavonoids including hesperetin and naringenin. Grapefruit oil contains: nonanal, nootkatone, beta-Pinene, alpha-phellandrene, 3-carene, ocimene, octanol, trans-linalool oxide, cis-p-mentha-2, 8-dien-1-ol, alpha-pinene, limonene, linalool, Geraniol, citronellal, alpha-terpineol, neral, dodecanal, and alpha-humulene [10].

PHARMACOLOGICAL ACTIVITIES

Anti HIV

The 6, 7-dihydroxy-bergamottin of *Citrus paradisi* enhances bioavailability of HIV protease inhibitor (saquinavar) by

inhibiting cytochrome P450 iso-enzyme 3A4 in liver and gut [11-12].

Anti-inflammatory effect

The antiinflammatory effect of *A. occidentale* stem-bark aqueous extract alone and in combination with grapefruit (*Citrus paradisi* Macf.) juice was investigated on fresh egg albumin-induced rat paw edema. Like diclofenac (100 mg/kg p.o.), aqueous extract of *A. occidentale* stem-bark (800 mg/kg p.o.) produced time-related, sustained and significant reduction ($p < 0.05$-0.001) of the fresh egg albumin-induced acute inflammation of the rat hind paw. However, the antiinflammatory effect of the plant extract was found to be approximately 8-15 times less than that of diclofenac. Coadministration of grapefruit juice (5 ml/kg p.o.) with *A. occidentale* stem-bark aqueous extract (800 mg/kg p.o.) or diclofenac (100 mg/kg p.o.) significantly potentiated ($p < 0.05$-0.001) the antiinflammatory effects of the crude plant extract and diclofenac on fresh egg albumin-induced rat paw edema [13].

Antiatherogenic

Naringenin belongs to the class of flavonoids called the flavanones. The flavanones are abundant in citrus fruits such as grapefruit (*Citrus paradisi*) and the oranges (*Citrus sinensis*). The role of naringenin and the related citrus flavanone hesperetin in the prevention and treatment of disease has recently received considerable attention, with particular interest in the use of these flavanones as anticancer and antiatherogenic compounds [14-15].

Antibacterial

The antiseptic and antibacterial properties of unripe *Citrus paradisi* and *Ananas sativus* have been equally reported [16]. Antibacterial potency and synergistic effect of crude aqueous and methanolic extracts of parts against multi-drug resistant *S. typhi* were investigated and compared. *Salmonella typhi* isolated from patients suffering from typhoid fever was tested against nine plant parts: unripe *Carica papaya* fruit, *Citrus aurantifoliia, Anana sativus*, *Citrus paradisi* etc. The antibacterial activities of the extracts, individually and in combination were determined using agar diffusion method and the minimum inhibitory concentration (MIC) carried out by agar dilution technique. The plant materials possessed antimicrobial activity with greater efficacy when used synergistically on the test organism [17]. A high activity of *Citrus paradisi* (grapefruit) oil to *P. larvae* with MIC 385.0 mg/L has also been reported [18]. Antibacterial activities of eleven essential oils against *Paenibacillus larvae* (15 field strains and the reference BCCM / LMG 9820 strain) were studied by the disk diffusion method and the method of serial dilutions in agar. The minimal inhibitory concentration (MIC) of essential oils was determined within 1%–0.015% v/v. Citrus essential oils showed the inhibitory effect with MIC ≥ 0.25–0.5% v/v for grapefruit oil [19]. Ethyl acetate extracts from acid less *Citrus paradisi* inhibited *A. fumigatus* TISTR 3180 with MIC values of 0.28 mg/ml and MFC values of 0.28 mg/ml, respectively [20]. Antibacterial activity of some fractions of *Citrus paradisi* peels extracted with hexane, chloroform, acetone and methanol were evaluated against different bacteria. The alcohol soluble fraction was found to possess maximum activity followed by hexane extract. Extracts were more effective against Gram-positive than Gram-negative bacteria [21]. The methanolic and ethanolic extracts of these plants were tested for their antimicrobial activity. Results showed that the highest antimicrobial activity was exhibited by the methanolic extracts of *Citrus paradisi* [22].

Antibacterial and antifungal activity of ethanolic extract of grapefruit (*Citrus paradisi* Macf.) seed and pulp was examined against 20 bacterial and 10 yeast strains. The level of antimicrobial effects was established using an in vitro agar assay and standard broth dilution susceptibility test. Ethanolic extract exhibited the strongest antimicrobial effect against *Salmonella enteritidis* (MIC 2.06% m/V). Other tested bacteria and yeasts were sensitive to extract concentrations ranging from 4.13% to 16.50% (m/V) [23]. The *Citrus paradisi* and Ficus carica were tested against pathogenic microorganisms; *S. aureus, E. coli, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans*. The extracts tested exhibited good antimicrobial activity against all the clinical isolates when compared with standard. The different extracts showed remarkable inhibitory action against various Gram positive and Gram negative bacteria and two fungal species. The methanolic, petroleum ether, chloroform, ethyl ether, ethanol extract of *Citrus paradisi* was screened for its antimicrobial activity. Antimicrobial activity was detected by observing the growth response of different organisms to the methanolic extract. It was generally based on the inhibition of growth of microorganisms which were measured with a desired concentration of the plant extract of *Citrus paradisi* to be examined with the standard concentration preparation [24].

Apoptotic activity

The aldehyde compounds of *Citrus paradisi* essential oil have been reported to induce apoptosis strongly in HL-60 cells [25].

Anxiolytic and antidepressant

Various extracts including petroleum ether, chloroform, methanol and water of the leaves of *Citrus paradisi* var. Duncan were tested using elevated plus maze (EPM) model and forced swimming test (FST) respectively in Swiss albino mice at different doses of extracts (i.e. 100, 200 and 400 mg/kg orally). Studies showed that methanolic extract at the dose of 100 mg/kg of the leaves of *Citrus paradise* var. Duncan markedly increased the average time spent in the open arms in EPM
and methanolic extract at the dose of 400 mg/kg showed a significant decrease in the time spent immobile [26]. Methanol extract at the dose of 100mg/kg of the leaves of *Citrus paradise* var. *foster* markedly increased the average time spent in the open arms in EPM and methanol extract at the dose of 400mg/kg showed a significant decrease in the time spent immobile by mice in FST [27].

The anti-anxiety activity of various extracts viz petroleum ether, chloroform, methanol and water, of the leaves of *Citrus paradisi* var. star ruby was evaluated using elevated plus maze (EPM) model in Swiss albino mice. Albino mice were treated orally with different doses of the extracts (i.e.100, 200 and 400 mg/kg) and behavior was observed on the EPM. Diazepam (2mg/kg, P.O) was used as a positive control. Methanol extract at the dose of 100mg/kg of the leaves of Citrus paradisi var. star ruby markedly increased the average time spent in the open arms of the EPM. This effect was comparable to the effect produced by diazepam [28-29].

Antioxidant

The antioxidant activity of bioactive compounds of grapefruit (*Citrus paradisi* Macf.) has been reported by using four *in vitro* models. The compounds selected include two limonoids, limonin and limonin 17-β-D-lucopyranoside; eight flavonoids, apigenin, scutelarein, kaempferol, rutin trihydrate, neoheseridin, neoeriocitrin, naringenin and aringin; and a coumarin bergapten. A variety of *in vitro*

models such as β-carotene, linoleic acid, DPPH, superoxide and hamster low-density lipoprotein were used to measure the antioxidant activity of 11 citrus bioactive compounds. The above compounds were tested at concentration of 10uM in all four methods. It was found that Limonoids, 17- β -D-lucopyranoside and bergapten inhibited 7%, whereas scutelarein, kaempferol and rutin trihydrate inhibited 51.3%, 47.0%, and 44.4%, respectively, using the β -carotene linoleate model system. Limonoids, 17- β -D-lucopyranoside, scutelarein, kaempferol, neoeriocitrin and rutin trihydrate showed 0.5% 0.25%, 32.2%, 18.3%, 17.2%, and 12.2%, respectively, free radical scavenging activity using the DPPH method. In the superoxide model, Limonoids, 17- β -D-lucopyranoside and bergapten inhibited the production of superoxide radicals by 2.5 10%, while the flavonoids such as scutelarein, neoeriocitrin and rutin trihydrate, and Neh inhibited superoxide formation by 64.1%, 52.1%, 48.3%, and 37.7%, respectively. However, 17-β-D-lucopyranoside did not inhibit LDL oxidation in the hamster LDL model. But, Limonoids and Bergapten offered some protection against LDL oxidation, increasing lag time to 345 min (3-fold) and 160 min (33% increase), respectively, while both Rutin and Neoeriocitrin increased lag time to 2800 min (23-fold). Scutelarein and kaempferol increased lag time to 2140 min (18-fold) and 1879 min (15.7-fold), respectively. In general, it seems that flavonoids, which contain a chromanol ring system, had stronger antioxidant activity as compared to limonoids and bergapten, which lack the hydroxy groups [30].

Caffeine metabolism

The effects of grapefruit juice and naringenin on the activity of the human cytochrome P-450 isoform CYP1A2 were evaluated using caffeine as a probe substrate. In vitro naringenin was a potent competitive inhibitor of caffeine 3-demethylation by human liver microsomes. *In vivo* grapefruit juice decreased the oral clearance of caffeine by 23% (95% CI: 7%-30%) and prolonged its half-life by 31% (95% CI: 20%-44%) (n = 12). It shows that grapefruit juice and naringenin inhibit CYP1A2 activity in man. However, the small effect on caffeine clearance *in vivo* suggests that in general the ingestion of grapefruit juice should not cause clinically significant inhibition of the metabolism of other drugs that are substrates of CYPIA2 [31].

Hematopoietic effect

The blood forming effects of (100% methanol seed extract) *Citrus paradisi* Macfad in adult Wistar rats for 30 days was a way of evaluating its traditional use in the treatment of blood deficiencies. Acute oral toxicity study was also conducted using limit dose test of the up and down procedure statistical program (AOT425PgmStat, Version 1.0) at a dose of 2000 mg/kg body weight/oral route. Results showed significant ($p<0.05$) progressive and dose dependent elevations in total leukocytes count, lymphocyte differentials, red blood count, hemoglobin concentration, packed cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet count. Reversed effect was recorded for the neutrophil and monocyte differentials which were significantly ($p<0.05$) decreased in the treated rats [32].

Hepatoprotective

The capacity of grapefruit juice (GJ) to inhibit the rate of micronucleated polychromatic erythrocytes (MNPE) in mice treated with benzo(a)pyrene (BaP), an environmental contaminant that is biotransformed by Cyp1a1 and is a strong

genotoxic agent. Administration of 4.1, 20.8 and 41.6 ul/g body weight of GJ to BaP-treated mice (340 mg/kg) a significant decrease in the frequency of MNPE at 48 and 72 h compared to BaP-only treated animals. In turn, no prevention of the cytotoxic damage induced by BaP was found. Whether GJ's antigenotoxic mechanism of action was related to an inhibitory effect on the activity of the Cyp1a1 enzyme. A reduction in microsomal hepatic and intestinal ethoxyresorufin-O-deethylase (EROD) activity of 20% and 44% respectively, was found in mice treated with BaP and GJ compared to BaP-only treated animals. Furthermore, when EROD inhibition was tested *in vitro,* a concentration dependent EROD inhibition by grapefruit juice, which reached 85% of the maximum level. Together, these researches suggest that the protective effect of GJ against the genotoxicity of BaP may be related to the inhibition of Cyp1a1 enzyme activity [33].

Arterial pressure

The coronary vasodilator and hypotensive effects of *Citrus paradisi* peel extract were assessed in the Langendorff isolated and perfused heart model and in the heart and lung dog preparation. In both models, *Citrus paradisi* peel extract decreased coronary vascular resistance and mean arterial pressure when compared with control values ($60 \pm 15 \times 10^7$ dyn s cm^{-5} vs $100 \pm 10 \times 10^7$ dyns cm^{-5} and 90 mmHg vs 130 ± 15 mmHg, respectively). These decreases in coronary vascular resistance and mean arterial pressure were blocked when isolated and perfused hearts and mongrel dogs were pre-treated with L-NAME. In humans, *Citrus paradisi* juice decreased diastolic arterial pressure and systolic arterial pressure both in normotensive and hypertensive subjects [34].

Antidiabetic effect

For this rats were used and divided into five groups. Rats were gavaged at the dose levels of 10ml/kg/day of distilled water, 10ml/kg of body weight/day of dimethyl sulphoxide (DMSO), 100, 300, and 600 mg/kg of body weight/ day of the extract dissolved in 10 ml/kg DMSO, respectively, for 30 days. On day 31, blood samples obtained were assayed for fasting plasma glucose (FPG), total cholesterol (TC), high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), and very low density lipoprotein (VLDL-c) using standard procedures. Cardiovascular disease risk assessing factors such as obesity or body mass index (BMI), atherogenic index (AI), coronary risk index (CRI) were calculated. Results showed significant ($p<0.05$, $p<0.001$) dose related lowering effects of the extract on FPG, cardiovascular disease risk assessing indices and lipid parameters except HDL-c fraction which was significantly ($p<0.05$, $p<0.001$) elevated. The extract also induced significant ($p< 0.05$) dose related weight loss in the treated rats in the latter 15 days of their treatment. These researches lend support to its therapeutic potentials in the management of suspected type-2 diabetic patients [35].

Other uses

In Sudan *Citrus paradisi* internal fruit peel is used to treat for malaria [36], gastro protective and antiulcer and this action is attributed to the antioxidant activity of citrus flavonoids found in grapefruit such as naringenin because this major flavonoid found exhibited the potent antibacterial and anti-helicobacter pylori activity *in vitro* and was also recently implicated in cytoprotection against injury induced by algal toxins in isolated hepatocytes [37-40]. Moreover naringenin, the bioactive component showed gastroprotective activity due to increase

expression of prostaglandins biosynthesis. Furthermore, it was shown to exhibit anti-cancer activity against human breast cancers. Therapeutic efficacy of citrus fruits such as red grapes and grapefruits is emphasized by the fact that they contain different classes of polyphenolic flavonoids, which were shown to inhibit platelet aggregation thus decreasing the risk of coronary thrombosis and myocardial infarction [41-44]. Citrus fruit peel is 1000 times sweeter than sucrose [45].

CONCLUSION

Current world-wide interest in traditional medicine has led to the rapid development and studies of many remedies employed by various ethnic groups of the world. Scientists from divergent fields are investigating new plants with an eye on their pharmacological usefulness. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole organism systems, including in particular toxicity studies as well as an examination of their effects in patients. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated regarding various disease treatment.

REFERENCES

1. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine 2007; 7: 463-9.
2. Ortuno A, Baidez A, Gomez P, Arcas MC, Porras I, García-Lidon A, Del Rio JA. *Citrus paradisi* and *Citrus sinensis* flavonoids: Their influence in the defence mechanism against *Penicillium digitatum*. Food Chemistry 2006; 98: 351-8.
3. Lucker J, Tamer MK, Schwab W, Verstappen FWA, Plas LHWV, Bouwmesster HJ, Verhoeven HA. Monoterpene biosynthesis in lemon (*Citrus lemon).* European Journal of Biochemistry 2002; 269: 3160-71.
4. Khan MN, Muhmad S, Baloch IH, Rehman S, Munawar MA. Characterization of essential oil of local varieties of *Citrus paradisi* Peel. J. of Chem. Soc. Pak. 2010; 32(5): 571-3.
5. Gupta V, Bansal P, Kumar P, Kaur G. Pharmacopoeial standards and pharmacognostical studies of leaves of *Citrus paradisi var. foster*. Research Journal of Pharmacognosy and Phytochemistry 2010; 3(2): 140-3.
6. Gupta V, Ghaiye P, Bansal P, Shri R. Pharmacopoeial standards and pharmacognostical studies of leaves of *Citrus paradisi Var. Duncan.* Journal of Pharmacy Research 2011; 4(4): 1084-6.
7. Del Rio JA, Fuster MD, Sabater F, Porras I, García-Lidon A, Ortuno A. Selection of citrus varieties highly productive for the neohesperidin dihydrochalcone precursor. Food Chem. 1997; 59: 433-437.
8. Pereira RMS, Andrades NED, Paulino N, Sawaya ACHF, Eberlin MN, Marcucci MC, Favero GM, Novak EM, Bydlowski SP. Synthesis and Characterization of a Metal Complex Containing Naringin and Cu, and its Antioxidant, Antimicrobial, Antiinflammatory and Tumor Cell Cytotoxicity. Molecules 2007; 12: 1352-66.

9. Kamal GM, Anwar F, Hussain AI, Sarri N, Ashraf MY. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. International Food Research Journal 2011; 18(4): 1275-82.
10. Bennett RD, Hasegava S, Herman Z, Glucosides of acidic limonoids in citrus. *Phytochemistry* 1989; 28: 2777-81.
11. Kupferschmidt HHT, Fattinger KE, Ha HR, Follath F, Krahenbuhl S. Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. Brit. J. Clin. Pharmacol. 1998; 45: 355-9.
12. Chinsembu KC, Hedimbi M. Ethnomedicinal plants and other natural products with anti-HIV active compounds and their putative modes of action. International Journal for Biotechnology and Molecular Biology Research 2010; 1(6): 74-91.
13. Ojewole JA. Potentiation of the antiinflammatory effect of *Anacardium occidentale* (Linn.) stem-bark aqueous extract by grapefruit juice. Methods Find Exp Clin Pharmacol 2004; 26(3): 183-8.
14. Samman S, Wall PML, Cook NC. Flavonoids and coronary heart disease: Dietary perspectives. In: Manthey JA, Buslig BS, eds. Flavonoids in the Living System. New York: Plenum Press; 1999. p. 469–81.
15. Wilcox LJ, Borradaile NM, Huff MW. Antiatherogenic Properties of Naringenin, a Citrus Flavonoid. Cardiovascular Drug Reviews 1999; 17(2): 160–78.
16. Alanis AD, Calzada F, Cervantes JA, Torres J, Ceballos GM. Antimicrobial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. Journal of Ethnopharmacology 2005; 100: 153-57.
17. Anthonia O, Olumide O. In Vitro Antibacterial Potentials and Synergistic Effect of South-Western Nigerian plant parts used in Folklore remedy for *Salmonella typhi* infection. Nature and Science 2010; 8(9): 52-9.
18. Fuselli SR, García de la Rosa SB, Eguaras MJ, Fritz R. Chemical composition and antimicrobial activity of Citrus essences on honeybee bacterial pathogen *Paenibacillus larvae*, the causal agent of American foulbrood. World Journal of Microbiology and Biotechnology 2008; 24: 2067–72.
19. Roussenova N. Antibacterial activity of essential oils against the etiological agent of American foulbrood disease (*Paenibacillus larvae*). Bulg. J. Vet. Med. 2011; 14(1): 17−24.
20. Chanthaphon S, Chanthachum S, Hongpattarakere T. Antimicrobial activities of essential oils and crude extracts from tropical Citrus spp. against food-related microorganisms. Songklanakarin J. Sci. Technol. 2008; 30(1): 125-31.
21. Negi P, Jayaprakasha G. Antibacterial activity of grapefruit (*Citrus paradisi*) peel extracts. European Food Research and Technology 2001; 213(6): 484-87.
22. Akroum S, Satta D, Lalaoui K. Antimicrobial, Antioxidant, Cytotoxic Activities and

Phytochemical Screening of Some Algerian Plants. European Journal of Scientific Research 2009; 31(2):289-95.
23. Cvetni Z, Aevi SV. Antimicrobial activity of grapefruit seed and pulp ethanolic extract. Acta Pharm. 2004; 54: 243–50.
24. Sharma MC, Sharma S. Phytochemical Screening and In vitro Antimicrobial Activity of Combined *Citrus paradisi* and *Ficus carica* Linn Aqueous Extracts. International Journal of Microbiological Research 2010; 1(3): 162-165.
25. Hata T, Sakaguchi I, Mori M, Ikeda N, Kato Y, Minamino M, Watabe K. Induction of apoptosis by *Citrus paradisi* essential oil in human leukemic (HL-60) cells. In Vivo 2003; 17: 553–59.
26. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activ ities of different extracts from *Citrus paradisi v*ar. Duncan. Asian Journal of Pharmaceutical an d Clinical Research 2010; 3(2): 98-100.
27. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activities of different extracts from *Citrus paradisi var. foster*. Journal Pharmacy Research 2009; 2(12): 1864-66.
28. Kamal M, Jawaid T. Herbal Drugs In Mirror of Anxiety Disorder -A Review. International Journal of Biomedical Research 2011; 2(1): 62-72.
29. Gupta V, Bansal P, Niazi J, Kaur G. Anti-anxiety Activity of *Citrus paradisi* var. star ruby Extracts. International Journal of PharmTech Research 2010; 2(3): 1655-57.
30. Jun YU, Wang L, Walzem RL, Miller EG, Pike LM, Patil BS. Antioxidant Activity of *Citrus* Limonoids, Flavonoids, and Coumarins. J. Agric. Food Chem. 2005; 53: 2009−14.
31. Fuhr U, Klittich K, Staib AH. Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. Br. J. clin. Pharmac. 1993; 35: 431-36.
32. Adeneye AA. Haematopoetic effect of methanol seed extract of *Citrus paradisi* Macfad (grape fruit) in Wistar rats. Biomedical Research 2008; 19(1): 23-6.
33. Alvarez-Gonzalez I, Mojica R, Madrigal-Bujaidar E, Camacho-Carranza R, Escobar-García D, Espinosa-Aguirre JJ. The antigenotoxic effects of grapefruit juice on the damage induced by benzo(a)pyrene and evaluation of its interaction with hepatic and intestinal Cytochrome P450 (Cyp)1a1. Food and Chemical Toxicology 2011; 49: 807–11.
34. Diaz-Juarez JA, Tenorio-Lopez FA, Zarco-Olvera G, del Valle-Mondragón L, Torres-Narvaez JC, Pastelín-Hernández G. Effect of *Citrus paradisi* extract and juice on arterial pressure both *in vitro* and *in vivo*. Phytotherapy Research 2009; 23(7): 948–54.
35. Adeneye AA. Methanol seed extract of *Citrus paradisi* Macfad lowers blood glucose, lipids and cardiovascular disease risk indices in normal Wistar rats. Nig Q J Hosp Med. 2008; 18(1):16-20.
36. Burham BO. Chemical Constituents of Selected Sudanese Medicinal and Aromatic Plants. A Thesis submitted to the Sudan

Academy of science in fulfillment of the Requirements for Master Degree in Chemistry 1984.
37. Reagor L, Gusman J, McCoy L, Carino E, Heggers JP. The effectiveness of processed grapefruit-seed extract as an antibacterial agent: An in vitro agar assay. J Altern Complement Med 2002; 8: 325-32.
38. Giamperi L, Fraternale D, Bucchini A, Rocco D. Antioxidant activity of *Citrus paradisi* seeds glyceric extract. Fitoterapia 2004; 75(20): 221-24.
39. Bae EA, Han MJ, Kim DH. In vitro anti-Helicobacter pylori activity of some flavonoids and their metabolites. Planta Med 1999; 65: 442-443.
40. Blankson H, Grotterod EM, Seglen PO. Prevention of toxin-induced cytoskeletal disruption and apoptotic liver cell death by the grapefruit flavonoid, naringin. Cell Death Differ 2000; 7: 739-46.
41. Motilva V, Alarcon de la Lastra C, Martin MJ. Ulcer-protecting effects of naringenin on gastric lesions induced by ethanol in rat: role of endogenous prostaglandins. J Pharm Pharmacol 1994; 46: 91-4.
42. So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. Nutr Cancer 1996; 26: 167-81.
43. Folts JD. Potential health benefits from the flavonopids in grappe products on vascular disease. Adv Exp Med Biol 2002; 505: 95-111.
44. Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR. Gastroprotective Effects Of Flavonoids In Plant Extracts. Journal of Physiology And Pharmacology 2005; 56(1): 219-31.
45. Surana SJ, Gokhale SB, Rajmane RA, Jadhav RB. Non –Saccharides natural intense sweeteners- An overview of current status. Natural product radiance 2006; 5(4): 270-8.