

Research Article

METHANOLIC EXTRACTION OF WHOLE DRIED FRUIT OF PUNICA GRANAATUM AND EVALUATION FOR ITS ANTI MICROBIAL ACTIVITY

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ABSTRACT

Plants have associate degree nearly limitless ability to synthesize aromatic substances, most of that area unit phenols or their oxygen-substituted derivatives. Most area unit secondary metabolites, of that a minimum of twelve, 000 are isolated, variety calculable to be but 100 percent of the overall. In several cases, these substances' function plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, like terpenoids, provide plants their odors; others (quinones and tannins) area unit liable for plant pigment. several compounds area unit liable for plant flavor (e.g., the terpenoid chemical irritant from chili peppers), and a few of an equivalent herbs and spices utilized by humans to season food yield helpful medicative compounds. In the present work an attempt was made to evaluate the anti-microbial activity of the Punica granatum, similar works have been performed by various scientists around the world but not used fruit as a whole extract in this work methanolic extracts of whole dried fruits are used and the results are found to be better than that of the extracts prepared from only seeds, peels, rind. The extracts from whole fruit showed better results but didn't cross the standard drugs results but by a minor margin.

KEY WORDS

Extracts, Punica granatum, methanolic, etc.

INTRODUCTION

Finding healing powers in plants is associate degree ancient plan. individuals on all continents have long applied poultices and imbibed infusions of a whole bunch, if not thousands, of native plants, qualitative analysis back to time period. there's proof that Neanderthals living sixty,000 years ago in current Iraq used plants like hollyhock; these plants square measure still wide employed in ethnomedicine round the world. traditionally, therapeutic results are mixed; very often cures or symptom relief resulted. Poisonings occurred at a high rate, also. Currently, of the one-quarter to simple fraction

of all prescription drugs distributed within the u. s. having higher-plant origins, only a few square measures meant to be used as antimicrobials, since we've relied on microorganism and plant life sources for these activities. Since the arrival of antibiotics within the Fifties, the utilization of plant derivatives as antimicrobials has been nearly nonexistent.

Clinical microbiologists have 2 reasons to have an interest within the topic of antimicrobial plant extracts. First, it's terribly seemingly that these phytochemicals can notice their method into the arsenal of antimicrobial medicine prescribed by physicians; many square

measures already being tested in humans. it's reportable that, on average, 2 or 3 antibiotics derived from microorganisms' square measure launched annually. when a worsening therein pace in recent decades, the pace is once more quickening as scientists understand that the effective lifetime of any antibiotic is proscribed. Worldwide disbursal on finding new anti-infective agents (including vaccines) is anticipated to extend hr from the disbursal levels in 1993 (7). New sources, particularly plant sources, also are being investigated. Second, the general public is turning into more and more conscious of issues with the over prescription and misuse of ancient antibiotics. additionally, many folks have an interest in having additional autonomy over their medical aid. a large number of plant compounds (often of unreliable purity) is quickly accessible over-the-counter from seasoner suppliers and natural-food stores, and self-medication with these substances is commonplace. the utilization of plant extracts, likewise as different kinds of medical treatments, is enjoying nice quality within the late Nineties. Earlier during this decade, around simple fraction of individuals surveyed within the u.s. used a minimum of one "unconventional" medical care throughout the previous year. it had been reportable that in 1996, sales of botanic medicines enlarged thirty seventh over 1995. it's speculated that the yank public could also be reacting to cover prescription of typically harmful medicine, even as their predecessors of the nineteenth century (see below) reacted to the overuse of hemorrhage, purging, and mercurous chloride.

Brief History

It is calculable that their square measure 250,000 to 500,000 species of plants on Earth. a comparatively tiny share (1 to 10%) of those

square measure used as foods by each human and different animal species. it's doable that even additional square measure used for medicative functions. Hippocrates (in the late fifth century B.C.) mentioned three hundred to four hundred medicative plants. within the 1st century A.D., Dioscorides wrote Delaware medicine, a medicative plant catalog that became the model for contemporary pharmacopoeias. The Bible offers descriptions of roughly thirty healing plants. Indeed, olibanum and myrrh most likely enjoyed their standing of nice value because of their medicative properties. reportable to own antiseptic properties, they were even used as mouthwashes. the autumn of ancient civilizations forestalled Western advances within the understanding of medicative plants, with abundant of the documentation of plant prescription drugs being destroyed or lost. throughout the historic period, the Arab world continued to excavate their own older works and to make upon them. Of course, Asian cultures were additionally busy collection their own collection. within the West, the Renaissance years saw a revival of ancient drugs, that was engineered mostly on plant Medicinals.

MAJOR GROUPS OF ANTIMICROBIAL COMPOUNDS FROM PLANTS

Plants have associate degree virtually limitless ability to synthesize aromatic substances, most of that are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of that a minimum of twelve,000 are isolated, variety calculable to be but 100 percent of the entire. In several cases, these substances' function plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, like terpenoids, offer plants their odors; others (quinones and tannins) are to blame for plant pigment. several compounds are

to blame for plant flavor (e.g., the terpenoid chemical irritant from chili peppers), and a few of identical herbs and spices utilized by humans to season food yield helpful healthful compounds.

Phenolics and Polyphenols

Simple phenols and phenolic acids.

Some of the best bioactive phytochemicals encompass one substituted synthetic resin ring. Cinnamic and caffeic acids are common representatives of a large cluster of phenylpropane-derived compounds that are within the highest number (Fig.1).

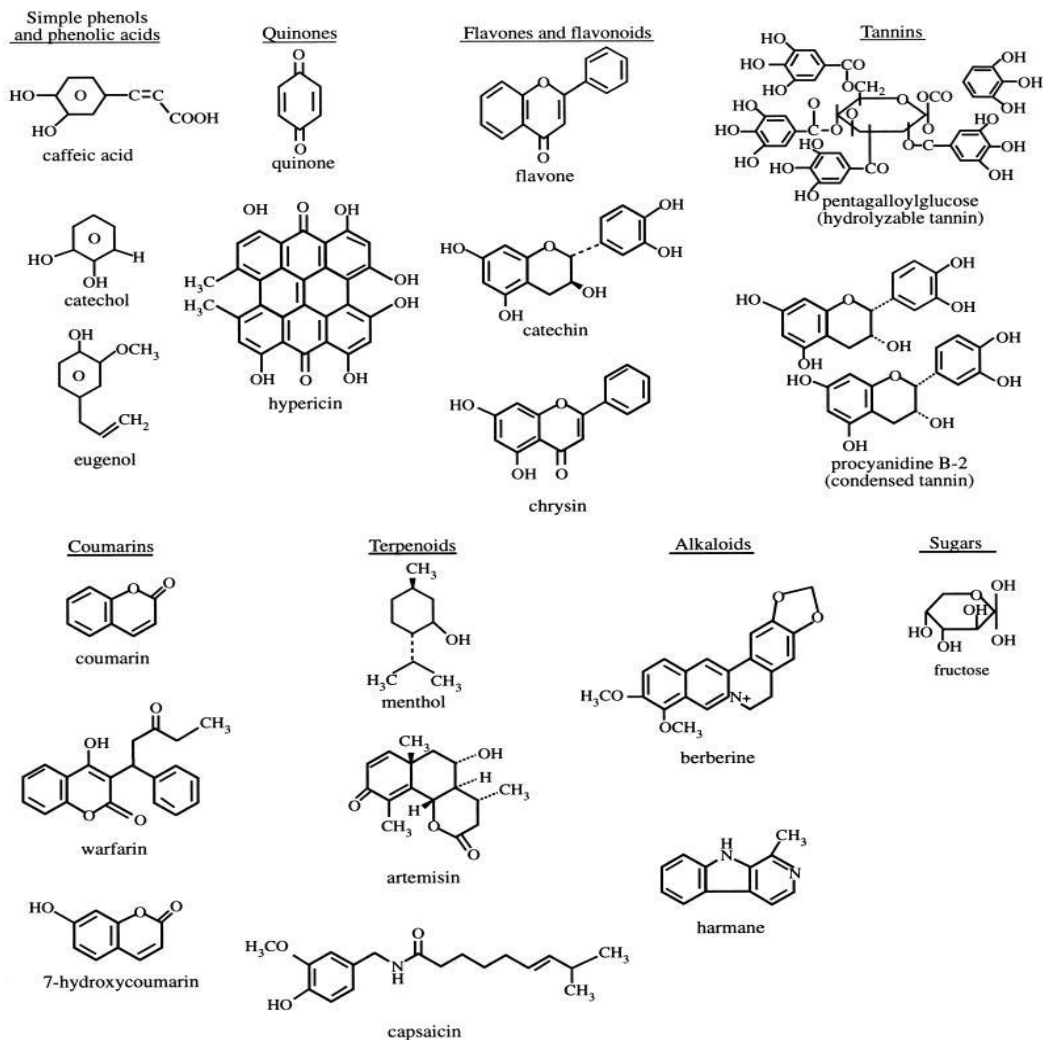


Fig 1: Structures of common antimicrobial plant chemicals.

The chew stick is wide utilized in African countries as associate degree oral hygiene aid (in place of a toothbrush). chew sticks return from completely different species of plants, and at

intervals one stick the with chemicals active element is also heterogeneous. Crude extracts of species used for this purpose, *Serindeia werneckii*, strangled the dental medicine

pathogens Porphyromonas gingivalis and bacteria genus melaninogenicus *in vitro*. The active element of the Nigerian chew stick (Fagara zanthoxyloides) was found to include varied alkaloids. whether or not these compounds, long utilised in developing countries, may realize use within the Western world isn't nevertheless known.

Papaya (*Carica papaya*) yields an opaque sap, usually known as a latex, that may be an advanced mixture of chemicals. Chief among them is enzyme, a widely known protease. associate degree organic compound, Carpaine, is additionally gift. Terpenoids are gift and will contribute to its antimicrobial properties. Osato et al. found the latex to be organic process to *B. subtilis*, *Enterobacter cloacae*, *E. coli*, *Salmonella typhi*, *staph aureus*, and *Proteus vulgaris*.

Ayurveda may be a kind of healing craft practiced in Bharat however not unknown within the u. s. Ayurvedic practitioners consider plant extracts, each "pure" single-plant preparations and mixed formulations. The preparations have lyrical names, like Ashwagandha (*Withania somnifera* root), Cauvery a hundred (a mixture), and Livo-vet. These preparations are won't to treat animals similarly as humans. additionally, to their antimicrobial activities, they need been found to own medication, immunomodulatory, anticancer, and mind-altering properties. *in vivo* studies of Abana, associate degree Ayurvedic formulation, found a small reduction in through an experiment evoked viscus arrhythmias in dogs. 2 microorganisms against that Ayurvedic preparations have activity are genus *Aspergillus* spp. and *Propionibacterium acnes*. (The aspergillosis study was performed with mice *in vivo*, and it's thus not possible to see whether or not the consequences are thanks to the stimulation of phagocyte activity within the

whole animal instead of to direct antimicrobial effects.)

The toxicity of Ayurvedic preparations has been the topic of some speculation, particularly since a number of them embrace metals. Prpic-Majic et al. known high levels of lead within the blood of adult volunteers United Nations agency had self-medicated with Ayurvedic medicines.

Propolis may be a crude extract of the balsam of varied trees; it's usually known as bee glue, since honeybees gather it from the trees. Its chemical composition is incredibly complex: just like the latexes represented on top of, terpenoids ar gift, similarly as flavonoids, carboxylic acid acids and esters, and substituted phenoplast acids and esters. artificial cinnamic acids, similar to those from propolis, were found to inhibit agglutination activity of respiratory disorder virus. Amoros *et al.* found that propolis was active against associate degree acyclovir-resistant mutant of HS1, animal virus kind two, stomatitis virus, and enterovirus. Mixtures of chemicals, like are found in latex and propolis, might act synergistically. whereas the flavone and flavonol elements were active in isolation against HS1, multiple flavonoids incubated at the same time with the virus were more practical than single chemicals, a attainable clarification of why propolis is more practical than its individual compounds. Of course, mixtures are a lot of probably to contain nephrotoxic constituents, and that they should be completely investigated and standardized before approved to be used on a large-scale basis within the West. Other Compounds

Many phytochemicals not mentioned on top of are found to exert antimicrobial properties. This review has tried to concentrate on reports of chemicals that are found in multiple instances to move. It ought to be mentioned, however, that there are reports of antimicrobial properties

related to polyamines (in specific spermidine), isothiocyanates, thiosulfinates, and glucosides. Polyacetylenes merit special mention. Estevez-Braun et al. isolated a C17 polyacetylene compound from *Bupleurum salicifolium*, a plant native to the island. The compound, 8S-heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol, was restrictive to *S. aureus* and *B. subtilis* however to not gram-negative microorganism or yeasts. ethyne compounds and flavonoids from plants historically utilized in Brazil for treatment of protozoal infection fever and liver disorders have additionally been related to antiprotozoal drug activity.

Much has been written regarding the antimicrobial effects of fruit crush. traditionally, girls are told to drink the juice so as to stop and even cure tract infections. within the early Nineteen Nineties, researchers found that the carbohydrate fruit sugar gift in cranberry and blueberry juices competitively strangled the surface assimilation of infective *E. coli* to tract animal tissue cells, acting as associate degree analogue for mannose. Clinical studies have borne out the protecting effects of fruit crush. several fruits contain fruit sugar, however, and researchers ar currently seeking a second active compound from fruit crush that contributes to the antimicrobial properties of this juice.

LITERATURE SURVEY

Ashraf A. Mostafa 2017, Prevention of food spoilage and food poisoning pathogens is usually achieved by use of chemical preservatives which have negative impacts including: human health hazards of the chemical applications, chemical residues in food & feed chains and acquisition of microbial resistance to the used chemicals. Because of such concerns, the necessity to find a potentially effective, healthy safer and natural alternative preservatives is increased. Within

these texts, Plant extracts have been used to control food poisoning diseases and preserve foodstuff. Antimicrobial activity of five plant extracts were investigated against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using agar disc diffusion technique. Methanolic extracts of *Punica granatum*, *Syzygium aromaticum*, *Zingiber officinales* and *Thymus vulgaris* were potentially effective with variable efficiency against the tested bacterial strains at concentration of 10 mg/ml while extract of *Cuminum cyminum* was only effective against *S. aureus* respectively. *P. granatum* and *S. aromaticum* methanolic extracts were the most effective plant extracts and showed bacteriostatic and bactericidal activities against the highly susceptible strains of food borne pathogenic bacteria (*S. aureus* and *P. aeruginosa*) with MIC's ranged from 2.5 to 5.0 mg/ml and MBC of 5.0 and 10 mg/ml except *P. aeruginosa* which was less sensitive and its MBC reached to 12.5 mg/ml of *S. aromaticum* respectively. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding health hazards of chemically antimicrobial agent applications.

K.L. Compean et al.,2014, The increasing incidence of microorganisms becoming resistant to antibiotics has continuously become a scientific community concern. Many scientists around the world are performing research on plants to be able to discover possible antimicrobial compounds. To date, many plant secondary compounds are known to have diverse biological activities. These may include antibacterial, antifungal and anticancer activities. In this regard, many scientists want to isolate, purify and identify plant secondary

metabolites. The aim of this study is to provide recent insights on the different secondary metabolites class and the experimental evidences of antibacterial activities against many different pathogens.

Jang-Gi Choi et al.,2011, Punica granatum is commonly used in Korea as a traditional medicine for the treatment of pathogenic bacteria. In this study, we investigated the in vitro and in vivo antimicrobial activity of P. granatum peel EtOH extract (PGPE) against 16 strains of Salmonella. The minimal inhibitory concentrations of PGPE were in the range of 62.5–1000 $\times 03BCg mL^{-1}$. In addition, the in vivo antibacterial activity of the PGPE extract was examined in a S. typhimurium infection mouse model. Mice were initially infected with S. typhimurium and then with PGPE. The extract was found to have significant effects on mortality and the numbers of viable S. typhimurium recovered from feces. Although clinical signs and histological damage were rarely observed in the treated mice, the untreated controls showed signs of lethargy and histological damage in the liver and spleen. Taken together, the results of this study indicate that PGPE has the potential to provide an effective treatment for salmonellosis.

Aim and objectives: The aim of the present research work is to evaluate the anti-microbial activity of the methanolic extract of punica granatum dried fruit powder.

- Collection of Fruits
- Preparation of Methanolic Extracts
- Extract is then is then distilled to remove solvent and used for testing antimicrobial activity.
- Test for Phytochemical Analysis includes Alkaloids, cardiac glucoside, Terpenoids,

reducing sugars, Saponins, phenol, Carbonyl, Flavonoids, Phlobatanin, Steroids

- Antimicrobial activity

PLANT PROFILE

PUNICA GRANATUM

The pomegranate (Punica granatum) may be a fruit-bearing deciduous ligneous plant or little tree within the magnoliopsid family that grows between five and ten m (16 and thirty-three linear unit.) tall.

The pomegranate originated within the region extending from modern Asian nation through Asian country and Asian nation to northern Asian nation, and has been cultivated since past throughout the Mediterranean region. it had been introduced into Spanish America within the late sixteenth century and into Golden State by Spanish settlers in 1769.

Description

A pomegranate shrub growing in the Draa River valley of southern Morocco

A shrub or small tree growing 5 to 10 m (16 to 33 ft) high, the pomegranate has multiple spiny branches and is extremely long-lived, with some specimens in France surviving for 200 years. P. granatum leaves are opposite or subopposite, glossy, narrow oblong, entire, 3–7 cm (1.2–2.8 in) long and 2 cm (0.79 in) broad. The flowers are bright red and 3 cm (1.2 in) in diameter, with three to seven petals. Some fruitless varieties are grown for the flowers alone.

Fruit, arils and seeds

Red-purple in color, the pomegranate fruit husk has two parts: an outer, hard pericarp, and an inner, spongy mesocarp (white "albedo"), which comprises the fruit inner wall where arils attach. Membranes of the mesocarp are organized as nonsymmetrical chambers that contain seeds inside arils, which are embedded without

attachment to the mesocarp. Containing juice, the arils are formed as a thin membrane derived from the epidermal cells of the seeds. The number of seeds in a pomegranate can vary from 200 to about 1,400.

Botanically, the edible fruit is a berry with seeds and pulp produced from the ovary of a single flower. The fruit is intermediate in size between a lemon and a grapefruit, 5–12 cm (2–5 in) in

diameter with a rounded shape and thick, reddish husk.

In mature fruits, the juice obtained by compressing the arils and seeds yields a sour flavor due to low pH (4.4) and high contents of polyphenols, which may cause a red indelible stain on fabrics. Primarily, the pigmentation of pomegranate juice results from the presence of anthocyanins and ellagitannins.

Pomegranate



Fruit of *Punica granatum* split open to reveal the clusters of juicy, gem-like seeds on the inside.

Scientific classification

Kingdom: Plantae
Clade: Angiosperms
Clade: Eudicots
Clade: Rosids
Order: Myrtales
Family: Lythraceae
Genus: *Punica*
Species: *P. granatum*

Binomial name: *Punica granatum* L.

Synonyms:

- *Punica florida* Salisb.
- *Punica grandiflora* hort. ex Steud.
- *Punica nana* L.
- *Punica spinosa* Lam

Chemical constituents:

Pomegranate peel: (i) Gallic acid (ii) Ellagic acid (iii) Punicalin (iv) Punicalagin (v) Caffeic acid (vi) Ilogitannins (vii) Pelletierine alkaloids (viii) Luteolin (ix) Kaempferol (x) Quercetin

Pomegranate juice

(i) Simple sugars (ii) Aliphatic organic acids (iii) Gallic acid (iv) Ellagic acid (v) Quinic acid (vi) Flavonols (vii) Amino acids (viii) Minerals (ix) EGCG (x) Ascorbic acid

Pomegranate root and bark (i) Ellagitannins (ii) Piperidine alkaloids (iii) Pyrrolidine alkaloid (iv) Pelletierine alkaloids

Pomegranate flower (i) Gallic acids (ii) Ursolic acid (iii) Triterpenoids (iv) Fatty acids

Pomegranate leaves

(i) Carbohydrates (ii) Reducing sugars (iii) Sterols (iv) Saponins (v) Flavanoids (vi) Tannins (vii) Piperidine alkaloids (viii) Flavone (ix) Glycoside (x) Ellagitannins

Pomegranate seed

(i) 3,3'-Di-O-methylellagic acid (ii) 3,3',4'-Tri-O-methylellagic acid (iii) Punicic acid (iv) Oleic acid (v) Palmitic acid (vi) Stearic acid (vii) Linoleic acid (viii) Sterols (ix) Tocopherols (x) Sex steroids

In vitro, in vivo and human trials had examined the effects of a range of pomegranate constituents on the prevention and reduction of atherosclerosis and LDL oxidation. Evidence suggested that polyphenolic antioxidants contained in pomegranate juice can cause reduction of oxidative stress and atherogenesis through the activation of redox-sensitive genes ELK-1 and p-JUN and increased e-NOS expression. Their results indicated that proatherogenic effects induced by disturbed shear stress can be reversed by constant administration of pomegranate juice. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduced

common blood pressure, LDL oxidation, and carotid intima-media thickness. Azadzo *et al.* demonstrated that 8-week administration of pomegranate juice concentrate daily in a rabbit model of arthritogenic erectile dysfunction significantly increased intra cavernous blood flow and smooth muscle relaxation, probably via its antioxidant effect on enhanced nitric oxide preservation and bioavailability. A pilot study in type 2 diabetic patients with hyperlipidemia found that concentrated pomegranate juice decreased cholesterol absorption, increased faecal excretion of cholesterol, had a favorable effect on enzymes concerned in cholesterol metabolism, drastically reduced LDL cholesterol, and improved LDL/HDL cholesterol and total/HDL ratios. Aviram *et al.* analyzed atherosclerotic lesion size, antioxidant activity, blood sugar, peritoneal macrophages, oxidative status, and lipid profiles for 3 months after giving 6 different pomegranate preparations with varying amounts of total polyphenols and gallic acid content in atherosclerotic apolipoprotein-E deficient mice and found that pomegranate phenolics and pomegranate unique complexed sugars could mimic the antiatherogenic effects of pomegranate extracts. All these evidences suggest the potential cardioprotective effect of pomegranate fruit.

MATERIALS AND METHODS

Collection of Fruits

The pomegranate was purchased from the local market. It was cut by making a shallow slit at the top of the pomegranate where the knob/stem is (this part is known as the crown). The shallow circle was created by cutting all the way around the rind. The inner seeds were revealed by pulling the crown of pomegranate. Three shallow slits were made by cutting the outer rind, followed by three of the white pith lines,

from the top to the bottom of the fruit. Three large sections were created by pulling the fruit. These sections were merged in a large bowl of cold water. The seeds were separated from the rind and pith. The seeds were drained in a colander to remove any additional pith that is mixed with the seeds.

Preparation of Methanolic Extracts

Methanolic extract (ME) was prepared by cutting fruit into small squares (approximately 5 mm²) which was dried at 55°C for 24 hours, and stored in an air tight container in the dark until further use, 10g each of the dried material is taken in a sample. It is then extracted with methanol using Soxhlet apparatus for 8hrs.

Extract is then is then distilled to remove solvent and used for testing antimicrobial activity.

Microorganisms

Type strains were obtained from American Type Culture Collection (ATCC) and National Collection of Industrial Microorganisms (NCIM) as follows: Staphylococcus aureus (ATCC 11105), Staphylococcus epidermis (ATCC 25619), Klebsiella pneumonia (ATCC 9621), Pseudomonas aeruginosa (ATCC 25619) a Candida albicans (NCIM 3100) which were all obtained from the microbiology laboratory of Hubert Enviro Care Systems Pvt Ltd. Each of the bacterial specimens was incubated in liquid culture dilutions and incubated at 37°C for 20 minutes to reach the logarithmic stage, then measured to a 0.5 Mc Farland dilution which delivered a final concentration of approximately 10⁵ CFU per ml. Then the agar plates with Methanolic and Aqueous extract of pomegranate were incubated over night at 37°C.

Test for Phytochemical Analysis

The extracts were analyzed for the presence of alkaloids, terpenoids, reducing sugars, saponins, tannins, carbonyls, flavonoids, phlobatannis and

steroids (Adetuyi et al., 2001; Trease and Evans, 1989; Sofowora, 1982).

Test for Alkaloids

Weigh concerning zero.2 weight unit of plant extract in separate tubing and warm with two% vitriol for two minutes. And it had been filtered in separate tubing and few drops of Dragencloffs chemical agent were additional and discovered for the presence of scarlet precipitates for the presence of alkaloids.

Test for cardiac glucoside

Keller-Killani check

Weigh concerning zero.5 weight unit of plant extract during a separate tubing with two milliliter of glacial ethanoic acid containing a drop of metal chloride resolution. This was underneath superimposed with one milliliter of targeted characin fish oxo salt (VI) acid. And observe for brown ring formation at the interface (Finar, 1983).

Test for Terpenoids

Weigh concerning zero.5 g plant extract in separate check tubes with two milliliter of chloroform. And add targeted vitriol rigorously to create a layer. And observe for presence of burnt sienna color interface to indicate positive results for the presence of terpenoids.

Test for reducing sugars

Take a tubing and add two milliliter of crude plant extract and add five milliliter of Distill water and filter. The filtrate was cooked with 3-4 drops of fehling's resolution A and B for two minutes. Observe for scarlet precipitate that indicates the presence of reducing sugars.

Test for Saponins

Weigh concerning zero.2 weight unit of plant extract within the tubing and add five milliliter of H₂O so heat to boil. Observe for the incidence of frothing (appearance of creamy mass of little bubbles) that then indicates the presence of glucoside.

Test for phenol

To little amount of plant extract was mixed with water and heated on water tub. The mixture was filtered and metal chloride was additional to the filtrate. And observe for dark inexperienced solutions that indicate the presence of a phenol.

Test for Carbonyl

Take two milliliter of plant extract in separate check tubes and add few drops two,4, di nitro phenyl reductant resolution and shake. And observe for the presence of yellow crystals directly for the presence of associate degree organic compound.

Test for Flavonoids

Weigh concerning zero.2 weight unit plant extract in separate check tubes and dissolved diluted hydrated oxide and add diluted coordination compound. And observe for yellow solutions that flip colorless. This indicates the presence of flavonoids

Test for Phlobatanin

Weigh concerning zero.5 weight unit of plant extract during a tubing and dissolve with H₂O and filter. The filtrate was cooked with two acid resolution. Observe for a red precipitate that shows the presence of Phlobatanin

Test for Steroids

To the plant extract add two milliliter of anhydride and add zero.5 weight unit of Methanolic extract of every sample with two milliliter of vitriol. Observe for the colour modification from violet to blue or inexperienced in samples indicating the presence of steroids

Antimicrobial activity

The antimicrobial potency of Methanolic extract of pomegranate on oral microorganisms was studied using disk inhibition method¹⁵. In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of each bacterium and fungi to yield a lawn of growth. After solidification of the agar, a number of sterilized disks were dipped into the solvents (negative controls) and extract solutions of different concentrations (4 μ g/ml, 8 μ g/ml, 12 μ g/ml and 100 μ g/ml) and placed on the plates. After incubation at 37°C for 24 h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks, streptomycin (100 μ g/ml) and Amphotericin-B (100 μ g/ml).

Interpretation of inhibition zones of test cultures was adopted from Johnson and Case 16. Diameter of zone of inhibition of 10 or less indicates test product being resistant to test organism, diameter zone of inhibition of 11 to 15 indicates test product being intermediate resistance to test organism, diameter zone of inhibition of 16 or more indicates test product being susceptible resistance to test organism.

RESULTS

After performing the preliminary tests for phytochemical constituents revealed the presence of following chemical constituents in Table No.1.

Preliminary Phytochemical Screening

Table 1: Preliminary Phytochemical Screening Results of *Punica granatum*

Phytochemical	Results
	<i>Punica granatum</i>
Steroid	-
Alkaloid	+
Tannin	+
Carbohydrate	+
Phenol	+
Flavonoid	+
Saponin	+
Reducing sugar	+
protein	+
Amino acid(free)	-
Terpenoids	+
Coumarin	+
(+) Present	(-) Absent

Table 2: Anti-microbial properties of Methanolic extracts of *Punica granatum* at four different concentrations against anti-microorganisms.

Pathogens	Extracts	Antimicrobial Activity*				Positive
		Conc of 4mg/ml	Conc of 8mg/ml	Conc of 12mg/ml	Conc of 100mg/ml	Conc of 100mg/ml
Staphylococcus aureus	ME	12	14	16	22	24
Staphylococcus epidermis	ME	12	15	16	8	21
Klebsiella pneumoniae	ME	6	6	6	8	25
Pseudomonas aeruginosa	ME	6	6	14	6	15
Candidaalbicans	ME	6	6	14	6	8

*Measured by the diameter of zone of inhibition in mm, Conc= Concentration,

**Streptomycin and Amphotericin-B are the positive control group

ANOVA indicated significant difference between all the Pomegranate extracts their Control

Thus, pomegranate fruit a saw hole has superior bioactivity against oral pathogens compared to its purified polyphenols, which illustrates the chemical synergy of the whole fruit's multiple compounds compared to single, purified, active ingredients.

CONCLUSION

In the present work an attempt was made to evaluate the anti-microbial activity of the punica granatum, similar works have been performed by various scientists around the world but not used fruit as a whole extract in this work methanolic extracts of whole dried fruits are used and the results are found to be better than



that of the extracts prepared from only seeds, peels, rind. The extracts from whole fruit showed better results but didn't cross the standard drugs results but by a minor margin.

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