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Antimicrobial and Antioxidant activity of Fruits (Papaya, Pomegranate and Guava)

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Abstract
The present study was designated to study the antimicrobial activities and antioxidant activity of aqueous and alcohol extracts that were prepared from fresh fruits of Carica papaya, Punica granatum, Psidium guava. The antimicrobial activities of the extracts were tested against bacteria *E.coil*, *P.aurignosa*, *S.aureus*, *B.subtiis* by the use of agar well diffusion method. Antioxidant activity were tested by Reducing power assay method, Superoxide anion scavenging activity, Total antioxidant capacity.

Keywords: antibacterial activity, antioxidant activity, punica granatum, psidium guajava, carica papaya

Introduction
There has been renewed interest in screening high plants for novel biologically active compounds, particularly those that effectively intervene the human ailments. There is a great demand of fruit juices in treatment of various illness such as arthritis, heart diseases and muscle aches and drug addiction (Tedesco *et al*., 2001) [22]. Even tens of thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is amazingly rapid (Jayaraman *et al*., 2008) [13]. Extraction of bioactive molecules from medicinal plants facilitates pharmacological studies leading to a synthesis of a more potent drug with a reduced toxicity (Beuchat et. al., 1994, Das *et al*., 1999) [4]. Plant based extracts can be extracted from any part of plant like barks, leaves, fruits and seeds etc**.** (Parekh and Chanda, 2007) [17]. The activity of various fruit extracts have been tested against various microorganisms like bacteria and fungi (Silvia *et al*., 2004) [21]. Generally Gram negative bacteria are more resistant than Gram positive bacteria (Rabe and Staden, 1997, Kelmanson *et al*., 2000, Parekh *et al*., 2005).
Carica papaya belongs to the fruits and vegetable class, it is highly abundant and is commonly known as pawpaw in Nigeria. About 30-100% of the fruits and vegetables are been wasted in Nigeria (Oluwalana, 2006). It is an invaluable plant that is prevalent throughout tropical Africa. Nigeria continues to be the third largest producer of papaya globally, and its level of production in Nigeria has been estimated to be 765,000 metric tonnes (FAO, 2007). Practically every part of the papaya plant is of economic value. Its uses range from nutritional to medicinal. Both fruit and seed of the ripe papaya are edible. The usefulness of fruits, roots and several parts of this plant has been largely reported with some minor negative effects (Onibon, 2007). The antimicrobial activity of various extracts prepared from pomegranate fruit peels were evaluated using both *in-vitro* agar diffusion and *in-situ* methods against some food-borne pathogens. It was found that 80% methanolic extract of peels was a potent inhibitor for *Yersini aenterocolitica, Listeria monocytogenes, Staphyllococcus aureus* and *Escherichia coli*. And the presence of active inhibitors in peels including phenolics and flavonoids were revealed by phytochemical analysis as potent constituents (Parashar *et al*. 2014) [18]
Psidium guajava Linn. is one of such medicinal plants belonging to the family Myrtaceae that is also used as a source of food. P. guajava has a rich ethno-medicinal history. Different parts of the plant are used in various indigenous systems of medicine, primarily for the treatment of gastrointestinal disorders (Begum *et al*., 2002; Jaiarj *et al*., 1999). Some of the ethno-medicinal uses includes the crushing of the leaves and the application of the liquids coming out from them on wounds, cuts, ulcers, boils, skin and soft tissue infectious site, rheumatic places (Aliyu, 2006). The guava tree is an evergreen small tree. The guava leaves are 2 to 6 inches long and 1 to 2 inches wide, aromatic when crushed, and appear dull-green with stiff but coriaceous with pronounced veins. There are bioactive components in the guava leaf that can fight against pathogens, regulate blood glucose levels, and can even aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances (Biswas *et al*. 2013) [5].
Pomegranate peel is recognized as a potential source of antioxidants for the stabilization of food systems. Presence of substantial quantities of phenolic compounds such as ellagic tannins, ellagic acids and gallic acids has been attributed to the antioxidant potential of pomegranate peel (Yasoubi *et al*., 2007; Afaf-haniem *et al*., 2010; Ibrahium, 2010) [23, 12].

Antimicrobial Activity
Materials and Methods for antimicrobial activity
Fruits samples and extract preparation
Punica granatum, Psidium guajava, Carica papaya were bought from the local market lucknow. Aqueous and alcoholic extracts were prepared by (Clarkson and Biby, 1969) [6].

Aqueous extract
The fruits is cut and crushed till it attains a roughage state .5gm og the ground/crushed fresh fruits is mixed with 100ml of distilled water in a sohxlet extraction apparatus for 4hrs at

100°C.Water extracts is prepared after running the extracts in sohxlet.

Alcoholic extracts

The fruits is cut and crushed to teh roughage extent and 5gm of the crushed or ground fresh fruit is taken mixed with 100ml of absolute alcohol. the mixture was agitated at room temperature for 8 hrs at room wrist action shaker. The mixture is allowed to stand for 12 hrs and alcohol is evaporated without heat. The residue is then mixed with 100ml of distilled water at 80°C.Alcohol extract is prepared.

Test microorganisms

The following microorganisms:

B. subtitles, S. aureus, E.coli, P. aeruginosa, were used for evaluating antimicrobial activity.

The bacterial stock cultures were incubated for 24h at 37°C o n Nutrient Agar.

Material and Methods

Antioxidant Activity

Distilled water, 2M phosphate buffer 1%, Potassium ferricynide, Trichloro acitic acid,

Pyrogallol, 6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate

HCL, 0.1% Fecl3, Eppendorf tube, Micropipette, Pipette tips and, Test tubes

Equipments

Centrifuge, Hot air oven and Spectrophotometer

Determinationation of Antimicrobial Activity

Antimicrobial activity was determined by Agar well diffusion method by Arora and kaur, 1999 [2].

Determination of antioxidant activity

Reducing power assay

The reducing power of the extract was determind as described by oyaizu (1986) [16]. The suspension of prepared extract 1ml of distilled water was mixed with 2.5 ml of 0.2M phosphate buffer (6.6) and 2.5 ml of 1% potasium ferricynide. The mixture was incubated at 50 degree for 20 min. Subsequently, 2.5ml of trichloro acetic acid was added and the mixture was then centrifuge at 3000 rpm for 10 min. A 2.5 ml alliquot of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% Fecl3, and the absorbance of the mixture was taken at 700 nm. A higher absorbance indicate a higher reducing power. In the assay, the colour of test solution changed to various shapes of green and blue, depending on the reducing power of each extract.

Super Oxide ION Scavenging Activity

Method of Markulund *et al*. (1974) [9] modified by Ekanayake *et al*. (2004) [8] was used for detremination of superoxide ion scavenging activity. This method is based on the inhibition of the autooxidation of pyrogallol by phenolic compound To the assay mixture composed of a phosphate buffer solution (2.6ml,50mM on water, pH8.22 +0.03) with the analytical prepared extract (0.3ml) was added a freshly prepared solution of pyrogallol (0.1ml of a 3mM solution of pyrogallol in 0.010M HCL (37.5%)).The oxidation reaction rate of pyrogallol was detrmined at 400nm by monitoring the absorbance every 30 second for a total periods of 10 min. corresponding to the end the reaction. The scavenging activity of the superoxide anion (O2) was calculated by the following formula (sun *et al*. 2001).

$$S = (K1-K2)/K1 \times 100$$

Where K1and K2 auxidation rates of the pyrogallol without and with the extract respectively.

Total Antioxidant Activity

Total antioxidant activity was determined by Shirwaikar *et al*. 2006 [20].

Result and Discussion of antimicrobial activity

Alcoholic extracts of Pomegranate shows better result in compare to papya and guava. (Table 1). Where aqueous extracts og guava shows higher inhibition zone as compared to pomegranate and papaya (Table 2).

Table 1: Alcoholic extracts of fruits

S No.	Fruits	E.coli (inhibition zone in mm)	P.aurignosa (inhibition zone in mm)	B.subtilis (inhibition zone in mm)	S.aureus (inhibition zone in mm)
1	Papaya	10mm	10mm	9mm	7mm
2	Pomegranate	30mm	11mm	11mm	10mm
3	Guava	10mm	6mm	8mm	10mm

Table 2: Aqueous extracts of fruits.

S.NO	Fruits	E.coli (inhibition zone in mm)	P.aurignosa (inhibition zone in mm)	B.subtilis (inhibition zone in mm)	S.aureus (inhibition zone in mm)
1	Papaya	9mm	7mm	9mm	7mm
2	Pomegranate	11mm	8mm	7mm	10mm
3	Guava	3mm	10mm	4mm	6mm

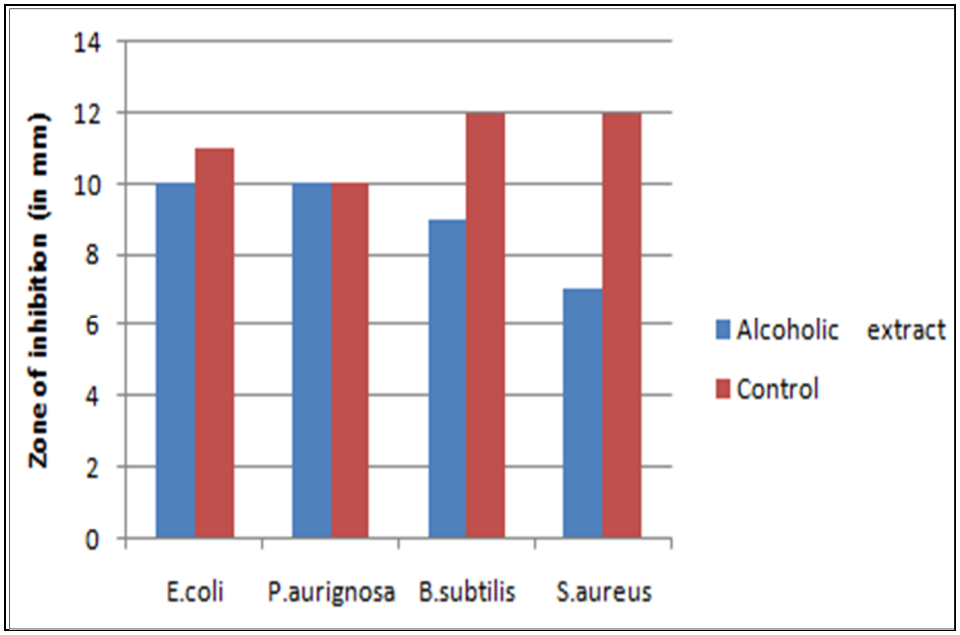

Fig 1: Antimicrobial activity of alcoholic extract of papaya

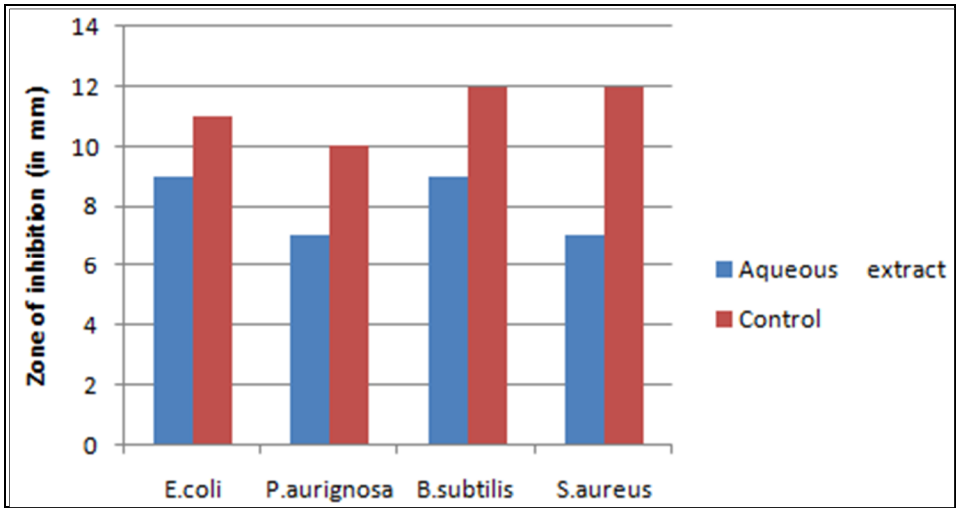

Fig 2: Antimicrobial activity of aqueous extract of papaya

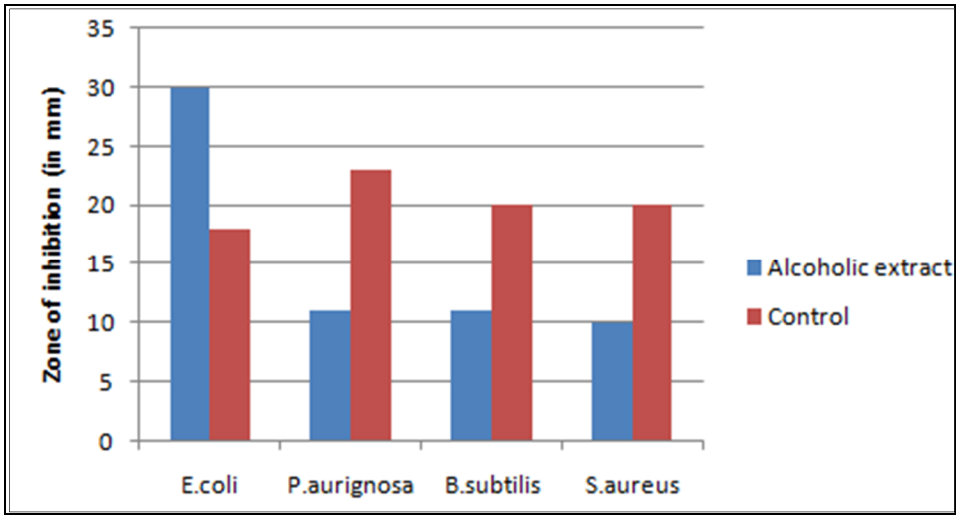

Fig 3: Antimicrobial activity of alcoholic extract of pomegranate

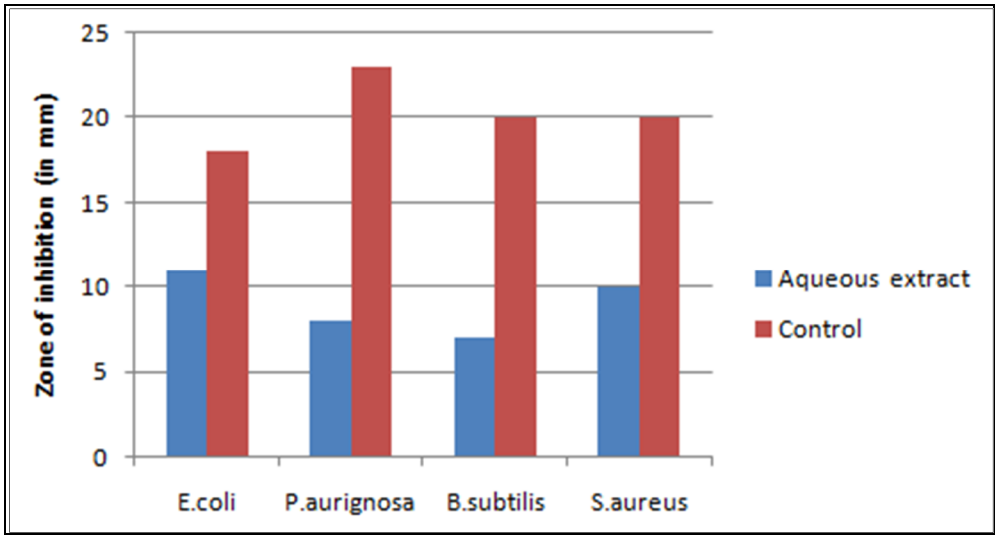

Fig 4: Antimicrobial activity of aqueous extract of pomegranate

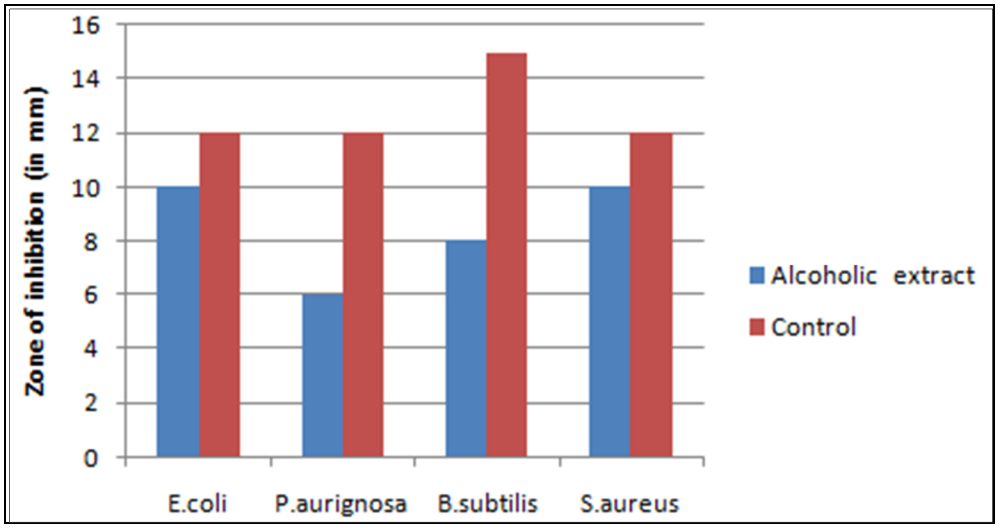

Fig 5: Antimicrobial activity of alcoholic extract of guava

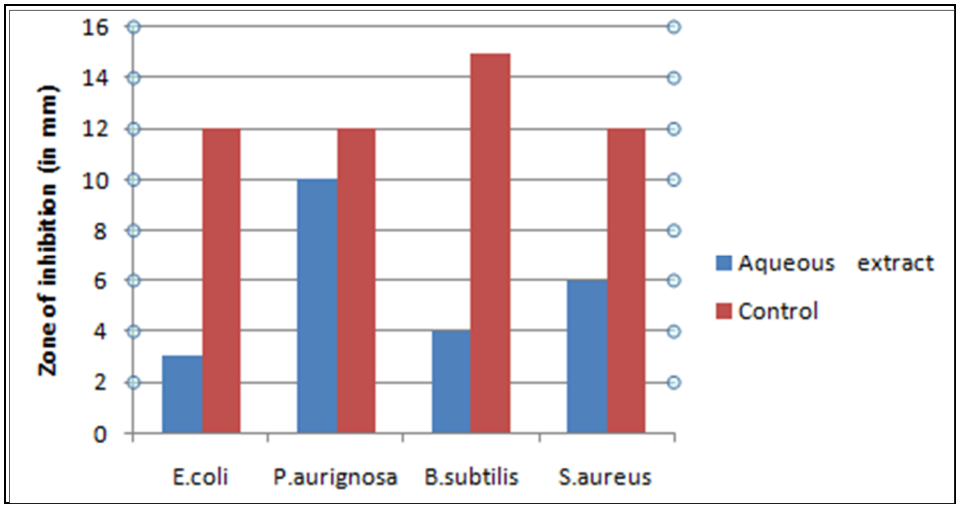

Fig 6: Antimicrobial activity of aqueous extract of guava

Alcoholic extract of pomegranate shows maximum zone of inhibition that is 30 mm against the strain of E.coli, where as aqueous extract of guava has shown maximum zone of inhibition 10 mm.

Result and Discussion of Antioxidant Activity

Reducing Power Assay

In accordance to Lobo *et al*., 2010 in the reducing power assay, the presence of antioxidants in the samples would

result in the reducing of Fe 3+ to Fe2+ by donating an electron. Amount of Fe2+ complex can then be monitored by measuring the formation of Perl's Prussian blue at 700nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. The aqueous extract of pomegranate shows maximum zone of inhibition that is 2.056.Where the aqueous extract of guava shows minimum inhibition zone 0.223.

Table 3: Reducing activity of fruits

Sample	Reducing activity
Papaya aq.	0.732
Papaya alc.	0.811
Pome granate aq.	2.056
Pome granate alc.	0.811
Gvaua aq.	0.223
Gvaua alc.	1.012

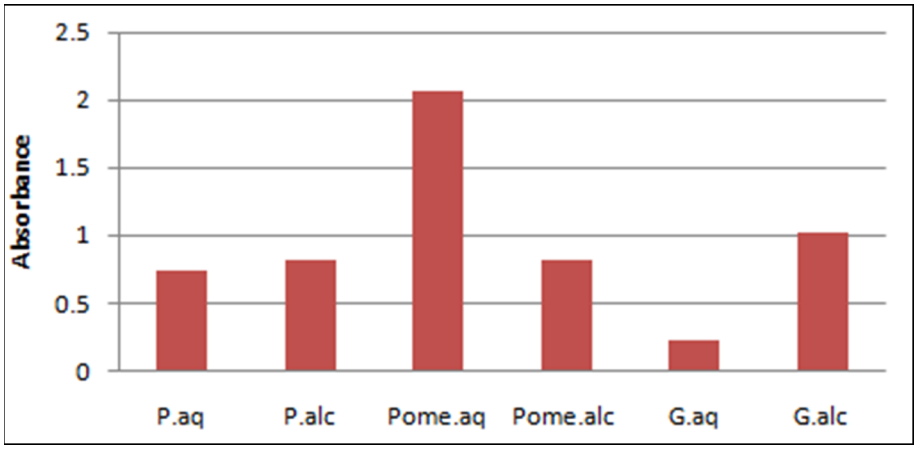

Fig 7: Reducing activity of extracts

Super anion scavenging activity

Superoxide radical a biologically quite toxic oxygen molecules with one pared electron and deployed by the immune system to kill the invalding microorganism, but also deleterious to cellular macromolecules on the other hands. Although superoxide anion was a weak oxidant, it gives rise to the generation of powerfull and dengerous hydroxyl radicals as well as singlet oxygen, both of which contribute to the oxidative stress and leads to the genesis of several chronic diseases in human beings. In super oxide scavenging methods dioxygen from super oxide anions O2 by a single electron transfer during the pyrogallol autooxidation in basic solution. The superoxide anions are scavenged by antioxidant and consequently, decrease the rate of pyrogallol autooxidation or even inhibit it. The result showed that aqueous and alcoholic extract of Papaya, Pome granate and Gvaua. Alcoholic and aqueous extract of pomegranate shows maximum scavenging activity at a extract concentration of 0.2%and 0.4% that is 2.056 and 2.012 repectively.

Table 4: Superoxide anion scavenging activity of fruits

Sample	Extract concentration (0.2%)	Extract concentration (0.4%)
Papaya aq.	0.732	0.723
Papaya alc.	0.811	0.806
Pome granate aq.	2.056	2.012
Pome granate alc.	0.811	0.806
Gvaua aq.	0.223	0.216
Gvaua alc.	1.012	1.014

Total antioxidant activity

The extracts demonstrated electron donating capacity as radical chain terminators, transformating reactive freeradical species. Alcoholic extract of guava shows maximum total antioxidant activity that is 2.74.

Table 5: Total antioxidant activity of fruits

Name of extract	Total antioxidant capacity
Papaya aq.	1.73±1.69
Papaya alc.	2.42±1.69
Pome granate aq.	1.23±1.69
Pome granate alc.	1.49±1.69
Gvaua aq.	2.32±1.69
Gvaua alc.	2.74±1.69

Conclusion

It is known that fruits are important source of sugar, minerals, organic acid, dietary fibre that have wide range of action which includes antibacterial, antiviral, antimicrobial, cardioprectective and antimutagenic activity. This study focuses on the possibility of using fruits as a source of natural antimicrobial and antioxidant activity.

The hazardous effect of synthetic antioxidant and the emergence of antibiotic resistant strains have revived the search for antioxidant and antimicrobial agents from natural sources. Different studies conducted on fruits, it had been found fruits holds a tremendous potential to serve as a source of effective, safer and better antimicrobial and antioxidant agent.

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