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COMPARATIVE ANALYSIS OF THE BIOLOGICAL ACTIVITIES OF BIO-INSPIRED GOLD NANO-PARTICLES OF *PHYLLANTUS EMBLICA* FRUIT AND *BETA VULGARIS* BAGASSE WITH THEIR CRUDE EXTRACTS

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Abstract

Over the last few decades, the appearance of the resistant strains as a result of the increased exploitation of antibiotics has challenged the researchers to develop improved techniques. One such technique makes use of the green approach for synthesizing metallic nano-particles. The characteristic, small size and the large surface area to volume ratio of these nano-particles mark their potential to fight against these pathogens. Two types of gold nano-particles (AuNPs) were synthesized using the aqueous extract of *Phyllanthus emblica* fruit and *Beta vulgaris* bagasse with tetra-chloroauric acid ($HAuCl_4.3H_2O$) as a metal precursor. Biogenic AuNPs so synthesized were investigated for their antimicrobial potential in relation to activities of the aforementioned plant materials in crude methanolic, ethanolic, acetone and aqueous extracts. The antioxidant and haemagglutination activities were also studied.

Keywords: Antibacterial, antifungal, anti-oxidant, haemagglutination, *Phyllanthus emblica, Beta vulgaris*.

Introduction

Plants having at least one of their parts (stem, roots, bark, leaves or fruit) used for curative and restorative purpose is termed as medicinal plants (Bruneton, 1993). This healing power of the plants corresponds to the presence of the important bioactive constituents that provide a base for the pharmacological activities in the human body (Akinmoladun *et al*., 2007). Several plants have already shown the biological activities that can be used for welfare of human beings (Shah *et al.,* 2014). These phytochemicals were categorized into two types; primary and secondary metabolites. Primary metabolites include amino acids, proteins, chlorophyll and sugars while essential oils, tannins, alkaloids, flavonoids, saponins and phenolic compounds were grouped together in secondary metabolites (Krishnaiah *et al*., 2009; Krishnaiah *et al*., 2007; Edeoga *et al*., 2005).Variety of the plants are studied, but still there are 25,00,000 species of higher plants in the world, of which the majority of the plants are not yet studied in relation to their pharmacological activities (Jeevam *et al*., 2004). *P. emblica*; a wonder herb owes its importance as 'King of Rasyana' (rejuvenation) and is frequently used ingredient in Indian Ayurveda because it represents a phytochemical reservoir for its significant medicinal applications. The fruit is richly supplied with tannins, flavonoids, gallic acids, vitamin C and other phyllemblic and polyphenolic compounds. Presence of terpenoids, flavonoids, alkaloids and tannins corresponds to its biological importance. Thus, *P. emblica* exhibits anti-genotoxic, anti-inflammatory, antioxidant, antitumor and anti-carcinogenic activities (Ekta *et al*., 2011). Similarly, *B. vulgaris* has been an important oriental ingredient in traditional Iranian pharmacognosy. Roots were used as diuretic, expectorants and for the treatment of liver diseases and mental troubles. Leaves were used as diuretic, tonic and soothe the inflammation, paralysis and liver and spleen disorders (Kirkitar & Basu, 1935; Khare, 2007). Leave of the plant also contained β-cyanins (red-violet pigments) and β-xanthines (yellow pigments), flavonoids, polyphenols, vitamins and minerals (Asolkar *et al*., 1992). While on one end, pharmacognosy was thriving to play an influential role in discovering new bioactive constituents from the plants, the emergence of new field; nanotechnology made its way to augment the role of the phytochemistry. Nanotechnology has gained much importance in the recent decades for the generation of nano-particles owing to their characteristic small size, distinct physiochemical capabilities and diverse applications in multidisciplinary fields. Variety of techniques is devised to produce eco-friendly product with less hazardous effects (Nie & Emory, 1997). One of the recent approaches followed now-a-days is green technology that makes use of plants and their fruit extracts for nano-particles synthesis (Begum *et al*., 2009; Bar *et al*., 2009; Parasher *et al*., 2009). Since metallic nano-particles produced through this route are more stable, biologically safe and less toxic in comparison to already existing chemical and physical procedures (Yu, 2007; Mallick *et al*., 2005; Liu & Lin, 2004; Smetuna *et al*., 2005). Plants and their extracts contains key ingredients like terpenoids and flavenones that reduce Au^{+3} to form AuNPs; serving both as reducing and capping agents for nano-particles synthesis (Shankar *et al*., 2004). In accordance with Raveendran, choice of solvent, non-toxic synthesizing material and use of reducing agent; that is environmentally safe and non-hazardous; are the three characteristic features, important for stability of nano-particles synthesized (Raveendran *et al*., 2003). The synthesized AuNPs served as good antimicrobial agents (Mohamed *et al*., 2010). Also larger surface area increases their potency against bacteria (Sun *et al*., 2005).

Material and Methods

Collection of plant material: *Phyllanthus emblica* fruit and *Beta vulgaris* bagasse were collected from Peshawar District and Dera Ismail Khan District, respectively Khyber Pakhtunkhwa (KPK), Pakistan. The plants were by Mr. Ghulam Jelani, Department of Botany, University of Peshawar, KPK, Pakistan.

Extraction and fractionation: 50gram of the shade dried powdered plant material was soaked for 15 days in 500ml of organic solvents (ethanol, methanol and acetone) in separate bottles at room temperature with occasional shaking. After 15 days, the samples were filtered and concentrated by using rotary evaporator to obtain the crude methanolic, ethanolic and acetone extract of *P. emblica* and *B. vulgaris bagasse***.**

Synthesis of biogenic gold nanoparticles: Green biogenic AuNPs were synthesized by using aqueous fruit extract of *P. emblica*. 500 ml of distilled water was added to 25 g of powdered fruit and boiled for 30 min. The extract obtained was filtered through Whatman No.1 filter paper. The aqueous extract was centrifuged twice at 10,000 rpm for 15 min at 4°C to remove cell debris. The resultant supernatant was further filtered through 0.2µm filter and employed for the fabrication of AuNPs. For the reaction to take place, the plant's aqueous extract was mixed with 1mM $HAuCl_4$. $3H_2O$ solution in 5: 95 ratio. Appearance of purplish red colour in a few minutes indicated the reduction of Au ions to AuNPs by the *P. emblica* fruit extract. The mixture was subsequently centrifuged at 10,000 rpm for 15 min at 4°C and re-dispersed to remove any unbound phytochemicals (Bsavegwda *et al*., 2013). The reaction mixture was then subjected to shaking water bath for one hour, concentrated by rotary evaporator and dried. Same procedure was followed for *B. vulgaris* bagasse and dark brownish color appeared in the reaction vessel when aqueous extract was subjected to $HAuCl_4$. $3H_2O$ solution confirming the reduction of AuNPs.

Anti-bacterial activity

Determining percent inhibition: Anti-bacterial activity was performed as per reported procedure of Bashir *et al*., 2010. Test organisms used in the study were *Escherichia coli*, Vanomycin resistant *staphylococcus aureus* (VRSA), *Proteus vulgaris, Morgnella morganii,* Methicillin resistant *Staphylococcus aureus* (MRSA), Methicillin susceptible *Staphylococcus aureus* (MSSA), *Pseudomonas aeruginosa* and *Rhodococcus rhodochrous.* The test microorganisms were cultured and refreshed on nutrient agar plates. Test organisms were inoculated in nutrient broth at 37°C for 24 hours. In the meantime, the autoclaved nutrient agar media was transferred to sterile petri plates and allowed to cool. On solidification, the plates were incubated for 24 hours at 37°C for sterility check. After the sterility check, the test organisms inoculated in nutrient broth were streaked with the help of sterilized culture swab on the plates and 6 mm borer was used to make the wells for the test samples. Test samples were prepared at concentration of 3mg/ml in DMSO (<1%). 100µl of the test sample was introduced into the respective well with the help of micropipette. Standard drug (Impenim) and DMSO were used as positive and negative controls, respectively. Plates were the left undisturbed for 3-4 hours for better diffusion and then incubated at 37°C for 24 hours. Zone of inhibition and percent inhibition was calculated by using formula.

$$\% \text{ inhibition} = \frac{\text{Zone of inhibition of sample}}{\text{Zone of inhibition of standard}} \times 100$$

Determination of minimum inhibitory Concentration (MIC): Minimum inhibitory concentration (MIC_{50}) was determined as per reported procedure of Bashir *et al*., 2010. The nutrient broth medium was prepared, autoclaved and 4 ml of the media was poured into test tubes. The sterility was checked and 18-24 hours old test strains were inoculated into the test tubes. Stock solution (5 mg/ml) was prepared for each sample in DMSO. Different dilutions (50, 100, 150, 200, 250, 300, 350, 400, 450, 500 µl) were introduced from the stock solution into the labeled test tubes containing test strains. The test tubes were then incubated at 37°C for 24 hours. The results were calculated on the basis of the turbidity and percent clarity in the test tubes.

Determination of Minimum Bactericidal Concentrations (MBC): Minimum bactericidal concentration (MBC) is the least effective amount of the antibiotic needed to kill a specified bacterium (Kaya *et al*., 2012; Hosgor *et al*., 2011). On the basis of the percent clarity in the test tubes, the test tubes were selected for the determination of MBC. Nutrient agar plates were made and labeled. For each sample, the wire loop was dipped in the clear test tube and streaked onto the respective compartment on the nutrient agar plate. The plates were then incubated for 24 hours at 37°C. The dilution of each sample showing no growth was considered to be its MBC value for that sample.

Antifungal activity: Antifungal activity was performed as per reported procedure of Bashir *et al.,* 2011. Test organisms used in current study were; *Penicillium digitatum, Verticilium dhalea, Aspergilus parasiticus, Aspergilus flavus, Fusarium oxysporum, Candida albicans* and *Aspergilus niger.* Stock solution was prepared for test samples at concentration of 24 mg/ml in DMSO. Sabouraud dextrose agar (SDA) medium was prepared and slants were prepared by adding 4ml aliquots of SDA media to sterilized test tubes. 66.67 µl from the stock solution was transferred to the test tubes and after solidification of slants, the test tubes were streaked with the fresh culture of test organism(s).The flucanazole and DMSO served as positive and negative control, respectively. Test tubes were incubated at 25°C for 7 days and results were noted according to the linear growth observed. Percent inhibition was determined by the formula:

$$\% \text{ inhibition} = 100 - \frac{\text{Growth in sample}}{\text{Growth in negative control}} \times 100$$

Antioxidant activity: Antioxidant activity was performed in accordance with the reported procedure (Aqil & Mehmood, 2006; Kannat & Sharma, 2007). Stock solution was prepared using 0.01g of each extract dissolved in 1ml of methanol and further dilution was done to make three concentrations (100, 200, 300µg/ml). 1ml of each concentration was mixed with 2 ml of freshly prepared DPPH solution. After incubation in dark room for 10 min, absorbance of each sample was determined by UV-Visible spectrophotometer at 517 nm for determining the antioxidant activity of the samples by the free radical scavenging effect of DPPH. The control includes 1ml methanol + 2ml DPPH solution and served as standard. Percent inhibition was determined by the formula.

$$\% \text{ inhibition of DPPH activity} = \frac{A_0 - A_1}{A_0} \times 100$$

where

A_0 = represents the absorbance of the control sample,

A_1 = represents the absorbance of the test sample (Khalil & Gabbiesh, 2009; Karca & Arslan, 2008).

A curve of percent inhibition or percent scavenging effect against samples concentrations was plotted and on the concentration where the scavenging reaches to 50% is its EC_{50} value.

Haemagglutination activity: The haemagglutination activity was performed against human erythrocytes of all blood groups according to the procedure of Naqvi *et al.,* 1992. Fresh blood was collected from healthy person and centrifuged to separate RBCs. RBCs suspension (2%) was prepared in phosphate buffer (pH 7). 1ml of RBCs suspension was mixed with 1 ml of each dilution of test sample (1:2, 1:4, 1:8 and 1:16).The test tubes were incubated for 30 min at room temperature. Rough granular deposition indicated a positive activity while smooth button formation illustrated negative result, the intensity of which was determined from the extent of deposition observed.

Results and Discussions

Antibacterial activity: Pakistan has a rich culture of using plants as a source for the medication purpose but still its rural population is dependent on the local systems for their health related issues (Khattak *et al*., 1985). At present, one of the problems that medical science facing is the antibiotic resistance; this aims to develop antimicrobials to resolve the health related issues (Freeman, 1997).

The antibacterial activity of the test samples was performed against selected pathogens (Figs. 1 & 2). Table 1 illustrates the MIC_{50} and MBC of *P. emblica* fruit aqueous, crude extracts and *P. emblica* fruit derived AuNPs. Aqueous extract of *P. emblica* fruits showed good antibacterial activity (74.07% with MIC_{50}=0.5 mg/ml and MBC=2.1mg/ml) against *R. rhodochrous*, *P. vulgaris* (69.23% with MIC_{50}=1.0 mg/ml and MBC=3.0 mg/ml), *E. coli* (64% with MIC_{50}=1.1mg/ml and MBC=3.3mg/ml), *M. morganii* (62% with MIC_{50}=1.4mg/ml and MBC=3.6mg/ml) and *P. aeruginosa* (62% with MIC_{50}=1.5mg/ml and MBC=4.0mg/ml). Moderate activity was observed against MSSA (57.14% with MIC_{50}=2mg/ml and MBC=4.9mg/ml) and VRSA (51.28% with MIC_{50}=2.25mg/ml and MBC=4.2mg/ml) while low antibacterial effect (34.88% with MIC_{50}=4.2mg/ml and MBC=9.4mg/ml) was found against MRSA. The acetone extract *P. emblica* fruit possess significant activity against *P. aeruginosa* (92% with MIC_{50}=0.6mg/ml and MBC=1.7mg/ml), *R. rhodochrous* (85.18% with MIC_{50}=0.9mg/ml and MBC=2.4mg/ml), *P. vulgaris* (84.61% with MIC_{50}=1.3mg/ml and MBC=3.1mg/ml). The same extract showed good antibacterial activity against *M. morganii* (75.86% with MIC_{50}=1.6mg/ml and MBC=3.5mg/ml) and *E. coli* (68% with MIC_{50}=2.1mg/ml and MBC=4.5mg/ml), moderate activity was observed against VRSA (53.84% with MIC_{50}=3.0mg/ml and MBC=6.3mg/ml) and MSSA (42.85% with MIC_{50}=3.5mg/ml and MBC=7.1mg/ml). Low inhibitory effect was observed against MRSA (38% with MIC_{50}=3.9mg/ml and MBC=8.6mg/ml).Crude ethanolic extract of *P. emblica* fruits significantly inhibit the growth of *R. rhodochrous* (96.29% with MIC_{50}=1.25mg/ml and MBC=2.9mg/ml), *M. morganii* (86.20%with MIC_{50}=1.5mg/ml and MBC=3.6mg/ml). Good inhibitory effect was observed against *P. aeruginosa* (72% with MIC_{50}=1.8mg/ml and MBC=3.7mg/ml), *E. coli* (72%with MIC_{50}=1.9mg/ml and MBC=3.8mg/ml), MSSA (71.42%with MIC_{50}=2.1mg/ml and MBC=mg/ml4.5) and VRSA (66.66%with MIC_{50}=2.7mg/ml and MBC=5.9mg/ml). However, low activity was observed against MRSA (39.53%with MIC_{50}=5.2mg/ml and MBC=11.1mg/ml) and *P. vulgaris* (38.46%with MIC_{50}=5.6mg/ml and MBC=12.8mg/ml).

The methanolic extract of *P. emblica* fruits showed significant inhibitory activity against *P. vulgaris* (84.61% with MIC_{50}=1.25mg/ml and MBC=2.9mg/ml), good inhibitory activity was observed against *M. morganii* (75.86% with MIC_{50}=1.7mg/ml and MBC=3.8mg/ml), *R. rhodochrous* (74.07% with MIC_{50}=1.7mg/ml and MBC=3.8mg/ml), MSSA (68.57% with MIC_{50}=2.0mg/ml and MBC=4.3mg/ml), *P. aeruginosa* (64% with MIC_{50}=2.2mg/ml and MBC=4.8mg/ml) and VRSA (61.53% with MIC_{50}=2.6mg/ml and MBC=5.7mg/ml), moderate activity was observed against *E. coli* (52% with MIC_{50}=3.7mg/ml and MBC=7.9mg/ml) and low activity was observed against MRSA (39.53% with MIC_{50}=6.4mg/ml and MBC=13.6mg/ml).

P. emblica fruit derived gold nano-particles showed significant inhibitory activity against *P. aeruginosa* (80% with MIC_{50}=0.8mg/ml and MBC=2.1mg/ml), good activity was observed against *R. rhodochrous* (77.77% with MIC_{50}=1.1mg/ml and MBC=2.6mg/ml) and *E. coli* (76% with MIC_{50}=1.3mg/ml and MBC=3.0mg/ml). Moderate activity was shown against MRSA (58.13% with MIC_{50}=2.3mg/ml and MBC=5.0mg/ml), *P. vulgaris* (57.69% with MIC_{50}=2.3 mg/ml and MBC=5.1mg/ml), VRSA (51.28%with MIC_{50}=2.9mg/ml and MBC=6.4mg/ml), *M. morganii* (44.28% with MIC_{50}=3.7mg/ml and MBC=7.7mg/ml) and MSSA (42.85% with MIC_{50}=3.2mg/ml and MBC=6.9mg/ml).

Table 2 illustrates the MIC_{50} and MBC of *B. vulgaris bagasse* aqueous, crude extracts and *B. vulgaris* bagasse derived gold nanoparticles. *B. vulgaris* bagasse aqueous extract showed moderate activity against *R. rhodochrous* (55.55% with MIC_{50}=2.8mg/ml and MBC=5.9mg/ml),

MSSA (48.57% with MIC_{50} = 3.4 mg/ml and MBC = 7.0mg/ml), *E. coli* (48%with MIC_{50}=3.5mg/ml and MBC=7.3mg/ml), *P. aeruginosa* (48%with MIC_{50}=3.5mg/ml and MBC=7.3mg/ml) and low inhibition was shown against *M. morganii* (34.48% with MIC_{50}=4.9mg/ml and MBC=9.2mg/ml). The aqueous extract was found inactive against VRSA, *P. vulgaris* and MRSA. The acetone extract showed good activity against *E. coli* (60% with MIC_{50}=2.3mg/ml and MBC=4.9mg/ml), moderate activity against *R. rhodochrous* (55.55% with MIC_{50}=2.9mg/ml and MBC=6.4mg/ml), *M. morganii* (51.72% with MIC_{50}=3.0mg/ml and MBC=6.3mg/ml) and *P. vulgaris* (50% with MIC_{50}=3.1mg/ml and MBC=6.5mg/ml), *P. aeruginosa* (44% with MIC_{50}=3.9mg/ml and MBC= 8.0mg/ml), MSSA (42.85% with MIC_{50}= 4.1mg/ml and MBC=8.4mg/ml) and low activity was observed against VRSA (28.20% with MIC_{50}=6.4mg/ml and MBC=13.3mg/ml). The acetone extract was found inactive against MRSA. The ethanolic extract was found moderately effective against *E. coli* (56% with MIC_{50}=3.6mg/ml and MBC=7.4mg/ml), *R. rhodochrous* (55.55%with MIC_{50}=3.6mg/ml and MBC=7.5mg/ml), *M. morganii* (55.17% with MIC_{50}=3.6mg/ml and MBC=7.5mg/ml), *P. vulgaris* (5.0%with MIC_{50}=4.1mg/ml and MBC=8.3mg/ml),*P. aeruginosa* (48% with MIC_{50}=4.4mg/ml and MBC=10.0mg/ml) and MSSA (42.85% with MIC_{50}=5.1mg/ml and MBC=10.6mg/ml). No activity was shown by ethanolic extract against MRSA and VRSA Methanolic extract showed good activity against *P. vulgaris* (61.53% with MIC_{50}=2.9mg/ml and MBC=5.9 mg/ml) and *E. coli* (60%with MIC_{50}=3.1mg/ml and MBC=6.3mg/ml), moderate activity against *R. rhodochrous* (55.55%with MIC_{50}=3.4mg/ml and MBC=7.0mg/ml), *M. morganii* (55.17%with MIC_{50}=3.4mg/ml and MBC=7.0mg/ml), *P. aeruginosa* (48%with MIC_{50}=3.9mg/ml and MBC=7.9mg/ml) and MSSA (45.71%with MIC_{50}=4.3mg/ml and MBC=8.8 mg/ml) and low activity was observed against VRSA (35.89%with MIC_{50}=5.8mg/ml and MBC=12.1mg/ml) and MRSA (34.88%with MIC_{50}=6.1mg/ml and MBC=12.7mg/ml). *B. vulgaris* bagasse nano-particles showed significant activity against *P. vulgaris* (80.76% with MIC_{50}=1.0mg/ml and MBC=2.1mg/ml), *P. aeruginosa* (80% with MIC_{50}=1.0mg/ml and MBC=2.1mg/ml), good activity was observed against *R. rhodochrous* (74.07% with MIC_{50}= 1.3 mg/ml and MBC=2.7mg/ml), *M. morganii* (73.07% with MIC_{50}=1.3mg/ml and MBC=2.8mg/ml) and *E. coli* (64% with MIC_{50}=1.7mg/ml and MBC=3.5mg/ml). Moderate activity was shown against VRSA (58.97% with MIC_{50}=3.0mg/ml and MBC=6.2mg/ml), MSSA (54.28% with MIC_{50}=3.1mg/ml and MBC=6.3mg/ml) and MRSA (44.18%with MIC_{50}=3.8mg/ml and MBC= 7.9mg/ml).

Table 1. MIC_{50} and MBC (mg/ml) values of crude extracts and AuNPs of *P. emblica* fruit.

Strains	Aqueous		Acetone		Ethanolic		Methanolic		AuNPs	
	MIC_{50}	MBC	MIC_{50}	MBC	MIC_{50}	MBC	MIC_{50}	MBC	MIC_{50}	MBC
VRSA	2.25	4.2	3.0	6.3	2.7	5.9	2.6	5.7	2.9	6.4
P. vulgaris	1	3.0	1.3	3.1	5.6	12.8	1.25	2.9	2.3	5.1
P. aeruginosa	1.5	4.0	0.6	1.7	1.8	3.7	2.2	4.8	0.8	2.1
MSSA	2	4.9	3.5	7.1	2.1	4.5	1	2.9	3.2	6.9
M. morganii	1.4	3.6	1.6	3.5	1.5	3.6	1.7	3.8	3.7	7.7
E. coli	1.1	3.3	2.1	4.5	1.9	3.8	3.7	7.9	1.3	3.0
R. rhodochrous	0.5	2.1	0.9	2.4	1.25	2.9	2.0	4.3	1.1	2.6
MRSA	4.2	9.4	3.9	8.6	5.2	11.1	6.4	13.6	2.3	5.0

Table 2. MIC_{50} and MBC (mg/ml) values of crude extracts and AuNPs of *B. vulgaris* bagasse.

Strains	Aqueous		Acetone		Ethanolic		Methanolic		AuNPs	
	MIC_{50}	MBC	MIC_{50}	MBC	MIC_{50}	MBC	MIC_{50}	MBC	MIC_{50}	MBC
VRSA	------	------	6.4	13.3	------	------	5.8	12.1	3.0	6.2
P. vulgaris	------	------	3.1	6.5	4.1	8.3	2.9	5.9	1.0	2.1
P. aeruginosa	3.5	7.3	3.9	8.0	4.4	10.0	3.9	7.9	1.0	2.1
MSSA	3.4	7.0	4.1	8.4	5.1	10.6	4.3	8.8	3.1	6.3
M. morganii	4.9	9.2	3.0	6.3	3.6	7.5	3.4	7.0	1.3	2.8
E. coli	3.5	7.3	2.3	4.9	3.6	7.4	3.1	6.3	1.7	3.5
R. rhodochrous	2.8	5.9	2.9	6.3	3.6	7.5	3.4	7.0	1.3	2.7
MRSA	-------	------	------	------	-------	------	6.1	12.7	3.6	7.9

Antifungal Activity: Majority of the test samples screened against the test organism(s) showed no activity (Figs. 3 & 4). All of the crude extract and AuNPs of *P. emblica* were found inactive against *P. digitatum.* Moderate activity (45.88%) was possessed by the AuNPs of *P. emblica,* low inhibitory activity was shown by the acetone (13.97%) and aqueous (29.41%) extracts while the methanolic and ethanolic extracts were found inactive against *V. dahlea*. No activity was shown by the crude extracts and AuNPs of *P. emblica* against *A. parasiticus, A. flavus* and *A. niger*. Low inhibitory activity (25%) was observed for the AuNPs of *P. emblica* while rests of the test samples were found inactive against *F. oxysporum*. Against *C. albicans* AuNPs and ethanolic extract of *P. emblica* showed low inhibitory

action (20 and 6.86%, respectively) and the remaining test samples were inactive.

The acetone extracts of *B. vulgaris* in active against the entire test organisms. The aqueous extract showed low activity (10%) against *A. niger* while rest of the organisms showed resistant to the test sample. The crude methanolic extract showed low activity (8.88%) against *C. albicans* and was found inactive against the remaining test fungi. The ethanolic extract possesses low activity against *P. digitatum* (10%) and *V. dahlea* (10.9%) amongst the test organisms. The AuNPs of *B. vulgaris* was inactive against the test fungi and very low inhibition was shown against *F. oxysporum* and *A. niger*.

Antioxidant activity: DPPH is a free radical compound used to determine the radical-scavenging ability of the various sample(s). It is a stable free radical which dissolves in methanol producing purple color and shows characteristic absorption at 517 nm. When antioxidants donate protons to this radical, the purple color of the DPPH assay solution turns light yellow resulting in a decrease in absorbance. This decrease in absorbance is taken as a measure to determine the extent of radical scavenging (Ferreira & Pereira, 2007; Othman & Adenan, 2007; Kubola & Siriamornpun, 2008).These scavenging activities were directly proportional to the concentration of the test samples. As the concentration of the test samples increased the percent scavenging activity also increased, when the scavenging reached to 50%, was its EC value. Lower the EC_{50} value higher will be the antioxidant activity (Gheldof & Engeseth, 2002). *P. emblica* and *B. vulgaris* bagasse AuNPs showed good antioxidant activities (62.50 and 62.58%, respectively) at concentration; 600µl/ml. Similarly, all extract showed highest antioxidant activity at 600µl/ml. The aqueous extract of *P. emblica* and *B. vulgaris* bagasse showed good scavenging activity 74.16 and 71.25%, respectively. The acetone extract of *P. emblica* and *B. vulgaris* bagasse were 66.66 and 52.08% active scavengers. Crude ethanolic extract of *P. emblica* possess moderate (54.16%) while *B. vulgaris* bagasse showed good antioxidant activity (69.58%). Crude methanolic extract of *P. emblica* showed good scavenging effect (69.16%) and that of *B. vulgaris* was found to be moderately effective (55.00%) (Figs. 5 & 6).

On the other hand EC_{50}values were calculated. Among *P. emblica* fruit extract, ethanolic extract showed maximum IC_{50} of 509.17µg/ml and lowest activity was observed against aqueous extract (143.73µg/ml). While in *B. vulgaris* bagasse; aqueous extract showed lowest EC_{50} (235.98µg/ml) and highest EC_{50} (509.08µg/ml) was shown by the acetone extract (Table 3).

Haemagglutination activity: Haemagglutination activity is accredited to a group of proteins known as 'lectins' (Benevides *et al*., 1999). These lectins are worthy agents for the separation and characterization of glycoprotein and glycol-conjugates, study of cell differentiation and histo-chemistry of cells and tissues (Gabius & Gabius, 1993). Phytolectins are of supreme importance in health sciences because they help in studying the structural and functional role of cell surface carbohydrates (Sharon & Lis, 1972). However, in the current study, no haemagglutination activity was observed for the test samples; *P. emblica* fruit and *B. vulgaris* bagasse. No agglutination result suggests the absence of phytolectins (Table 4).

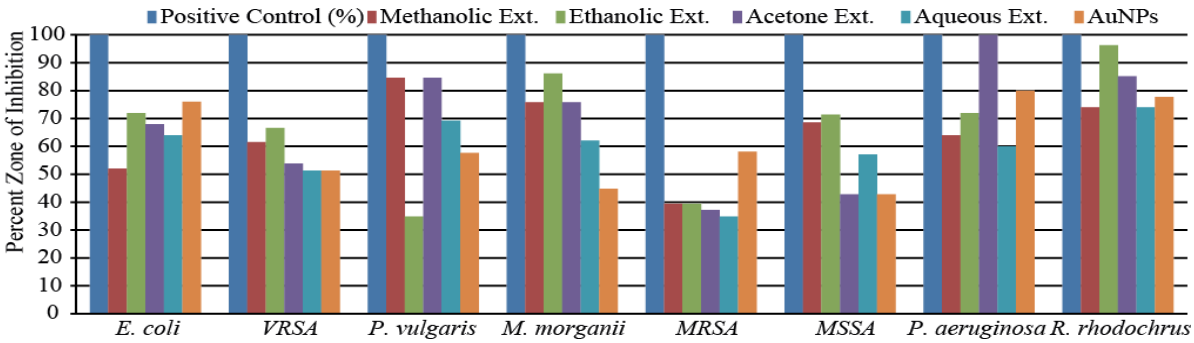

Fig. 1. Antibacterial activity of crude extracts and AuNPs of *P. Emblica* fruits.

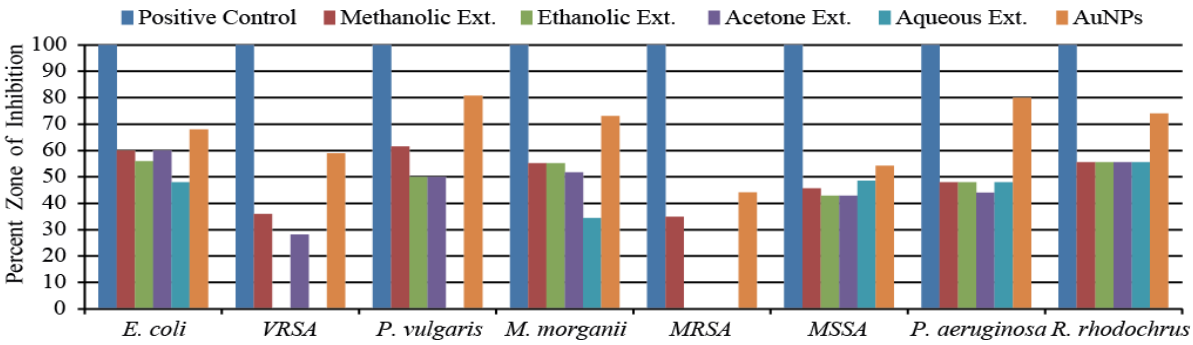

Fig. 2. Antibacterial activity of crude extracts and AuNPs of *B. Vulgaris* bagasse.

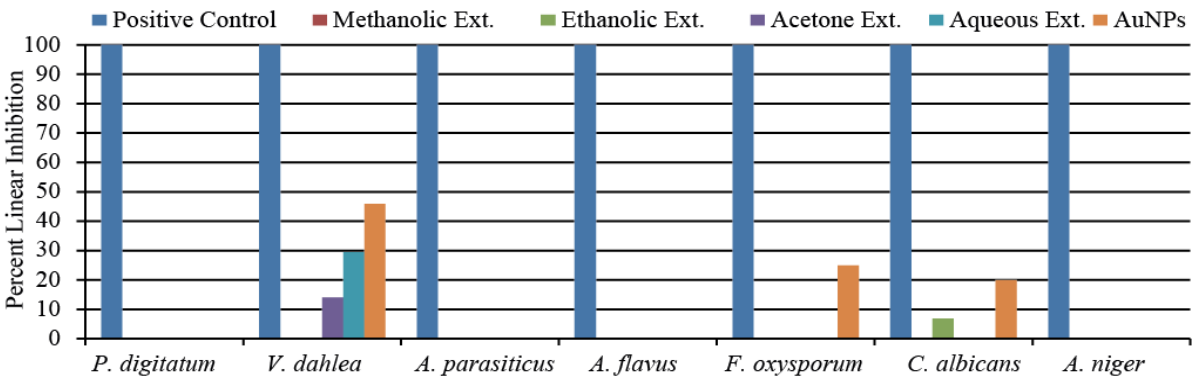

Fig. 3. Antifungal Activity of crude extracts and AuNPs of *P. emblica* fruits.

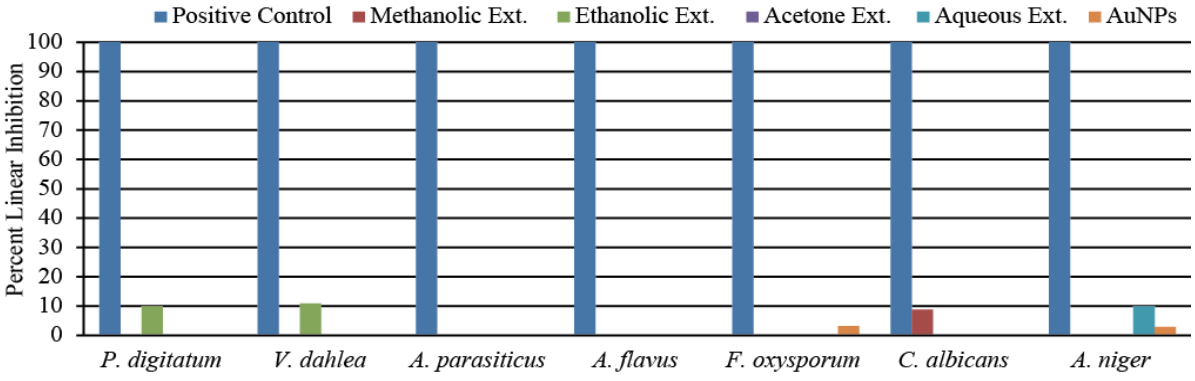

Fig. 4. Antifungal activity of crude extracts and AuNPs of *B. vulgaris* bagasse.

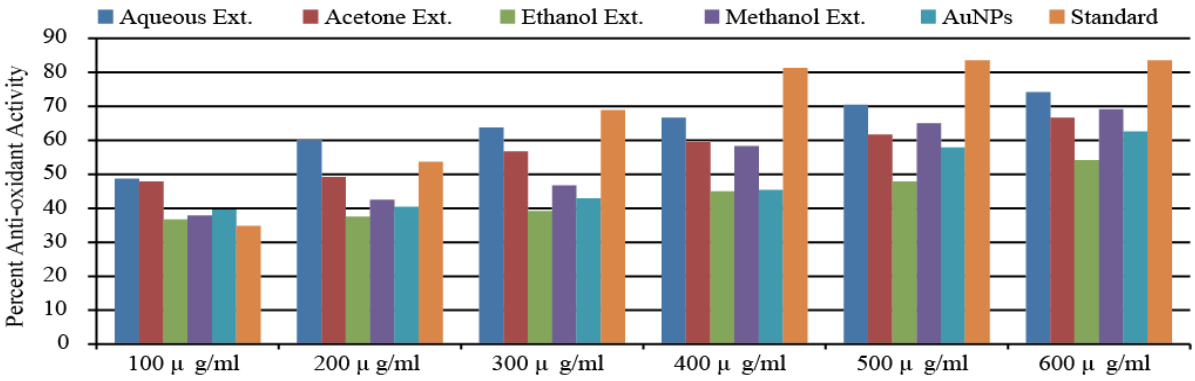

Fig. 5. Anti-oxidant activity of crude extracts and AuNPs of *P. emblica* fruits.

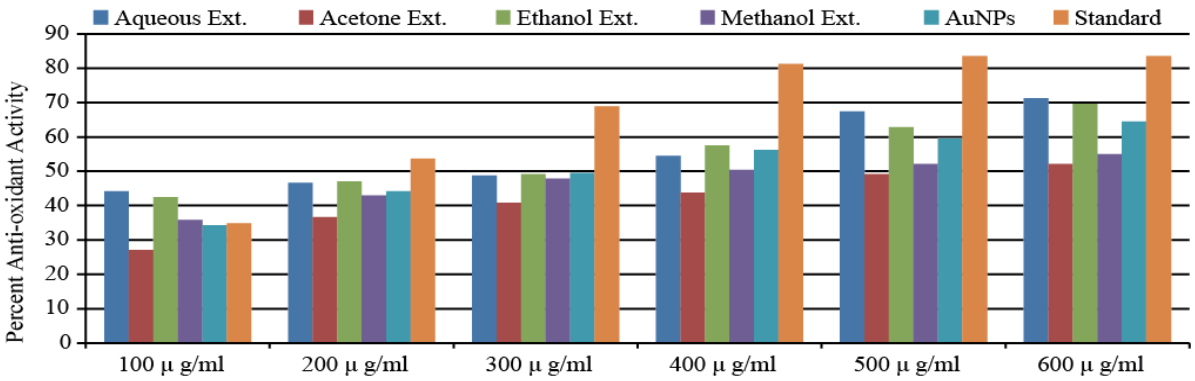

Fig. 6. Anti-oxidant activity of crude extracts and AuNPs of *B. vulgaris* bagasse.

Table 3. EC_{50} values of the crude extract and AuNPs of *P. emblica* fruits and *B. vulgaris* bagasse.

Test Sample(s)	EC_{50} (µg/ml)	Test Sample(s)	EC_{50} (µg/ml)
Crude extracts & AuNPs of *P. emblica* fruits		**Crude extracts & AuNPs of *B. vulgaris* bagasse**	
Methanol	295.26	Methanol	393.86
Ethanol	509.17	Ethanol	259.44
Acetone	164.64	Acetone	509.08
Aqueous	143.73	Aqueous	235.98
AuNPs	343.98	AuNPs	319.07
Standard	174.45	Standard	174.45

Table 4. Haemagglutination activity of Crude extract and AuNPs of *P. emblica fruits* and *B. vulgaris* bagasse.

Blood Group	Crude extracts and AuNPs of *P. emblica* fruits and *B. vulgaris* bagasse			
A+	1:2	1:4	1:8	1:16
A-	-	-	-	-
B+	-	-	-	-
B-	-	-	-	-
AB+	-	-	-	-
AB-	-	-	-	-
O+	-	-	-	-
O-	-	-	-	-

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