WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 2, 973-981. Research Article ISSN 2277– 7105

IN VITRO MICROPROPAGATION FROM NODAL EXPLANT OF KNOCK OUT ROSE- AND EVALUTION OF ITS ANTIBACTERIAL ACTIVITY

[1*]B. Janarthanam and [1]T. Sekar

Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai – 600 030, Tamil Nadu, India.

Article Received on
27 Nov. 2017,

Revised on 18 Dec. 2017,
Accepted on 08 Jan. 2018

DOI: 10.20959/wjpr20182-10704

***Corresponding Author**
B. Janarthanam
Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai – 600 030, Tamil Nadu, India.

ABSTRACT

A protocol has been developed for *in vitro* plant regeneration from a nodal explant of Knock out rose variety, an economically important variety for cut rose. The present study utilized 6-benzyl adenine (BA) and napthaleneacetic acid (NAA) for the induction of shoot organogenesis on nodal explants. The highest frequency of shoot regeneration (71.67± 2.9 %) and number of shoots per explant (8.00 ± 1.00) were obtained on medium supplemented with 4.44 µM BA and 0.14 µM NAA. Rooting was achieved on half strength MS solid medium supplemented with 2.46 µM indole-3-butyric acid (IBA) produced (6.0 ± 1.0) roots with an average height of 4.9 ± 0.26 cm after 30 days of culture. The rooted plantlets were transferred for hardening, 80 per cent of plantlets survived and resumed growth in the mixture of soil, vermiculite and farmyard manure (1:1:1). Antibacterial activity of ethanolic flower extract was measured by agar disc diffusion method. Different concentrations of ethanolic flower extract of Knock out rose was tested for the anti-bacterial activity. Ethanolic flower extract of Knock out rose showed the maximum zone of inhibition (17 mm) and (15 mm) against *Pseudomonas aeruginosa and Escherichia coli* respectively.

KEYWORDS: Knock out rose, Micropropagation, Nodal explant, Antibacterial activy.

INTRODUCTION

Rose is one of the most important ornamental crops and for centuries it is grown as greenhouse cut flowers production, pot-grown flowering houseplant sales and home

gardening market. Roses are one of the world's most popular flowers with more than 20,000 commercial cultivars, collectively based on only 8 of the approximately 200 wild species in Rosa (Roberts et al., 1990). Major constraints in rose improvement are: 1) conventional breeding methods are time consuming due to perennial nature of the crop; 2) high degree of sterility caused by discordant chromosome number and 3) infestation by pest, fungal (black spot, powdery mildew, dieback) and viral disease, thereby causing huge losses to the crop. Although propagation by vegetative means is a predominant technique in roses, yet does not ensure healthy and disease-free plants, moreover dependence on season and slow multiplication rate are some of the other major limiting factors in conventional propagation. The conventional method of propagation, like T–budding gives about 52 to 80% success during different months (Pati et al., 2006). As this method of propagation is cumbersome and time consuming, the alternative methodology practiced widely by researchers is the *in vitro* micropropagation method through tissue culture, which is quicker in bringing out more number of plantlets in a shorter period. Moreover the plantlets raised through tissue culture are free of contamination and diseases. Micropropagation protocols have been worked out well for ornamental roses, but only fragmentary information is available on micropropagation and regeneration of scented roses (Ishioka and Tanimoto, 1990; Koronova and Michailova, 1994). The main objective of this study was to establish a protocol for *in vitro* propagation for rapid and large scale production of disease free plants of Knock out Rose.

MATERIALS AND METHODS

Plant material: Healthy plants of Knock out Rose were collected from the Dr. M. S. Veeraraghavan (M.S.V) garden, Kodaikanal, Tamil Nadu, India and raised in pots containing soil, vermicompost and farm yard manure in the ratio of (1:1:1) under greenhouse conditions at PG & Research Department of Botany, Pachaiyappa's College, Chennai – 600 030.

Explant preparation: Explants were rinsed 3–4 times with sterilized double distilled water for 10 min. The cleaned explants were finally treated with 0.1 % $HgCl_2$ (w/v) for 4 min under aseptic conditions and again washed five times with sterile distilled water to remove traces of mercuric chloride ($HgCl_2$).

Shoot proliferation: The surface-sterilized nodal explants were trimmed to 0.8 cm and inoculated on MS medium supplemented with synergistic combination of BA (1.11, 2.22, 4.44 and 8.88 µM) and NAA (0.054, 0.14, 0.27 and 0.54 µM) for shoot multiplication. At the

end of the experiment, percentage of shooting, shoot length and the number of shoots per explant were recorded after 35 days in culture.

Rooting and acclimatization: The proliferated shootlets (5.0 cm in length) were excised and cultured on half strength MS medium containing various concentration of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM) for root induction. Root number and length were recorded after 30 days of culture. Well developed plantlets were rinsed thoroughly with sterile water to remove residuals and potted with a mixture of red soil, vermiculite and farm yard manure (1:1:1), covered with transparent polyethylene bags to ensure high humidity. After 15 days, the fully acclimatized plantlets were transplanted to plastic pots (80 mm diameter).

Culture conditions: MS basal medium containing 3% sucrose was used for all *in vitro* culture. The medium pH value was adjusted to 5.6 ± 0.2 prior to adding 0.9% agar (Himedia – CR301), media were autoclaved at 121°C for 15 min. The incubation conditions were 16/8 h light and dark cycle. The lighting was provided using white cool fluorescent tubes of 40 µmol/m^2/s light intensity at 25 ± 2°C.

Extract preparation: The Knock out rose flower was extracted with 75% of ethanol and then the extract was evaporated. The residue of extract was mixed with n-butanol and water (2:1) and both the upper layer of n-butanol and lower layer of water were separated and evaporated under vacuum. The residues were washed with petroleum ether to remove fatty components and then concentrated extract dissolved in ethanol (Shimizu et al., 1997). This extract used for antibacterial activity.

Inoculums preparation: Each bacterial strain was subcultured overnight at 35 °C in Mueller-Hinton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^{-7} CFU/ml using spectrophotometer. Antibacterial activity was measured using the standard method of diffusion disc plates on agar (Faten et al., 2014). Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth Medium (Hi media) for 24 hours at 37 °C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper disc (6mm in diameter) were placed on the agar medium to load 20µl of different concentrations (10, 20 and 30 mg/ml) of ethanolic flower extracts of knock out rose was tested. Inhibition diameters were measured after incubation for 24 hours at 37°C.

Statistical analysis: Each experiment was repeated three times and each treatment had six replicates. The data were analysed using analysis of variance (ANOVA) and means were compared using the Duncan's multiple range test (DMRT) using SPSS (SPSS version 16.0) at 5% level of significance ($p < 0.05$).

RESULTS AND DISCUSSION

Shoot proliferation from nodal explants cultured on MS medium supplemented with BA (1.11, 2.22, 4.44 and 8.88 µM) and NAA (0.054, 0.14, 0.27 and 0.54 µM) showed differential response according to the hormonal concentration used (Table 1). Cytokinins are known to exhibit a significant effect on shoot proliferation from nodal explants. The highest significance of shoot regeneration (71.67± 2.9%) and number of shoots per explant (8.00 ± 1.00) were obtained on medium supplemented with 4.44 µM BA and 0.14 µM NAA (Figure 1a, b, c). The various concentration and combinations of auxin and cytokinins showed organogenic differentiation has been well explained in plant tissue culture (Baskaran and Jayabalan, 2005; Gururaj et al., 2007; Janarthanam and Seshadri, 2008; Janarthanam and Sumathi, 2010).

The individual microshoots (5.0 cm) taken from the *in vitro* proliferated shoots were inoculated on half strength MS medium supplemented with various concentrations IBA for rhizogenesis. Among various concentration of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM), the maximum number of roots 6.00 ± 1.0 with 75.0 ± 5.0% response and an average root length of 4.90 ± 0.26 cm was obtained in half strength MS medium containing IBA (2.46µM). (Table 2: Figure 1d,e,f). Increasing the concentration of IBA (4.92 µM and 12.30 µM) resulted in a decrease in the percentage of root induction response with decrease number of roots and root length. Similar results have been reported by Asano and Tanimoto (2002), wherein healthy shootlets of miniature rose cultivar "Shortcake" were transferred to half strength MS medium with (2.46µM) and produced the best response for root proliferation.

Rooted planlets of Knock out rose were transferred to paper cups containing sterile soil, sand and vermiculite (1:1:1), kept one week and transferred to the shade house after 35 days of normal growth. Knock out rose plant, more than 75% of plantlets survived hardening in red soil, vermiculite and farm yard manure (1:1:1) for one week. However, the survival rate decreased by 15% after three weeks of transfer to soil. It was observed that very gradual acclimatization of *in vitro* grown plants to the external environment is most essential for Knock out rose plant. Sixty percent of the plants transferred to pots survived and resumed

growth (Fig. 1 g,h). The results showed the ability of the nodal explants to produce higher number of shootlets without any intervening callus phase, where all the plantlets were uniform in height and growth. Hence, we propose this protocol a simple, economical, rapid and highly reproducible to obtain more plantlets within a short period of time.

The ability of the ethanolic flower extracts of Knock out rose to inhibit the *bacterial* growth of selected bacterial organism was determined to various concentration (Table 3). The results revealed that the flower extracts showed antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* (Figure 2). Similar results were obtained on ethanolic peel extracts from *Punica granatum* which exhibited antibacterial activities (Janarthanam and Sumathi, 2015).

However, this study resolutely established the efficacy of the ethanolic flower extract of Knock out rose against known Gram-positive and Gram-negative bacteria, indicating that the plant produces compounds that are able to combat the bacterial defenses. Our results suggest that Knock out rose flower extract can serve as potential source of bioactive healthy compounds in the diet and their consumption could be useful in the prevention of diseases. Further research is needed towards isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

Table 1. Effect of different concentration of cytokinin (BA) and (NAA) in MS medium *in vitro* shoot multiplication from nodal explant of Knock out rose.

Plant growth regulator (μM)		**Shoot induction**	**Number of shoot per explant**	**Shoot length**
BA	NAA			
1.11	0.054	28.33 ± 2.9^{abcd}	1.67 ± 0.58^{ab}	1.40 ± 0.10^{a}
	0.14	40.00 ± 5.0^{eihg}	3.00 ± 0.00^{abc}	2.10 ± 0.20^{def}
	0.27	43.33 ± 5.8^{ihg}	4.00 ± 1.00^{c}	2.33 ± 0.15^{fg}
	0.54	21.67 ± 2.9^{a}	1.67 ± 0.58^{ab}	1.50 ± 0.10^{ab}
2.22	0.054	23.33 ± 5.8^{ab}	2.33 ± 1.15^{abc}	2.23 ± 0.06^{efg}
	0.14	33.33 ± 5.7^{bcde}	3.00 ± 1.00^{abc}	2.50 ± 0.20^{g}
	0.27	46.67 ± 2.9^{ih}	3.33 ± 1.15^{bc}	1.90 ± 0.10^{cde}
	0.54	31.67 ± 2.9^{abcde}	2.00 ± 0.00^{ab}	1.73 ± 0.12^{abc}
4.44	0.054	45.00 ± 8.7^{ihg}	2.67 ± 1.15^{abc}	1.40 ± 0.10^{a}
	0.14	**71.67 ± 2.9^{j}**	**8.00 ± 1.00^{dc}**	**4.37 ± 0.50^{h}**
	0.27	50.00 ± 5.0^{i}	2.67 ± 1.15^{abc}	2.53 ± 0.21^{g}
	0.54	38.33 ± 2.8^{deh}	3.00 ± 1.00^{ab}	1.43 ± 0.12^{a}
8.88	0.054	35.00 ± 5.0^{cdeg}	2.33 ± 0.58^{abc}	1.57 ± 0.21^{abc}
	0.14	38.33 ± 7.6^{dehg}	3.00 ± 1.00^{abc}	2.40 ± 0.10^{fg}
	0.27	31.67 ± 7.6^{abcde}	3.00 ± 1.00^{ab}	1.80 ± 0.20^{bcd}

	0.54	25.00 ± 8.6^{abc}	1.33 ± 0.58^{a}	1.73 ± 0.15^{abc}
F- value		14.892	8.714	44.115
P- value		0.000	0.000	0.000

Data were recorded after 35 days of culture. Results represent mean ± SD of six replicated experiments. Values denoted by different letters differ significantly at p<0.05 level using Duncan's multiple range test (DMRT)

Table 2. Effect of different concentration of auxin (IBA) in half strength MS medium on rooting response of Knock out rose.

IBA (µM)	Rooting response (%)	Number of rootlets / shoot	Root length (cm)
0.49	30.00 ±5.00^{a}	2.67 ±0.58^{a}	3.07±0.51^{b}
0.98	31.67 ±2.89^{a}	2.67 ±1.15^{a}	3.07±0.25^{b}
2.46	**75.00 ± 5.00^{c}**	**6.00 ±1.00^{b}**	**4.90±0.26^{c}**
4.92	45.00 ±5.00^{b}	2.67 ±0.58^{a}	2.30±0.30^{a}
12.30	36.67 ±2.89^{a}	2.00 ±1.00^{a}	2.00±0.20^{a}
F- value	55.772	9.500	36.218
P- value	0.000	0.001	0.000

Data were recorded after 30 days of culture. Results represent mean ± SD of six replicated experiments. Values denoted by different letters differ significantly at p<0.05 level using Duncan's multiple range test (DMRT).

Table 3: Antibacterial activity from flower extract of Knock out rose.

Micro-organisms Tested	Zone of inhibition (mm) Concentrations of extract		
Ethanolic flower extract	10mg/ml	20mg/ml	30mg/ml
Bacillus subtilis (MTCC No. 100224)	8	12	15
Bacillus cereus (MTCC No. 10221)	9	11	14
Pseudomonas aeruginosa (MTCC No. 14676)	9	12	17
Staphylococcus aureus (MTCC No. 9542)	9	10	12
Escherichia coli (MTCC No. 1563)	9	13	15

Inhibition Zone in diameter (mm)*

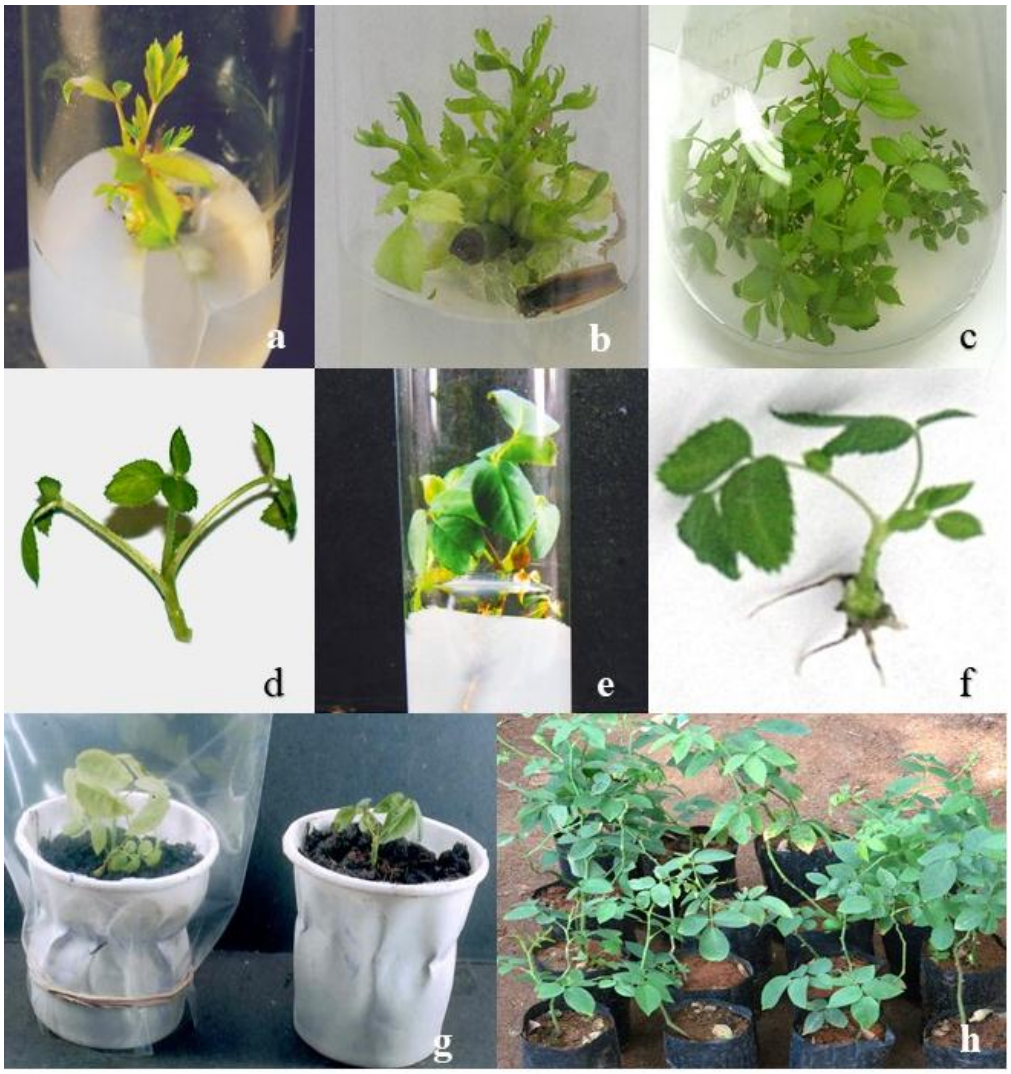

Figure 1. ***In vitro*** **micropropagation from nodal explant of knock out rose.**

(a) & (b) Initiation of shoots from nodal explant after three weeks of culture on MS medium supplemented with 4.44 µM BA and 0.14 µM NAA, (c) Proliferation of multiple shoots (8.00 ± 1.00) from shoot tip explants at 35 days of cultured on MS medium containing 4.44 µM BA and 0.14 µM NAA, (d, e) Rooted plantlets after 30 days of culture on ½ strength MS medium containing 2.46 µM IBA, (f) A well established plant, (g) Well established and hardened in vitro plants successfully transferred to the paper cups, (h) Hardened plants transferred to external environment condition showing luxurious growth.

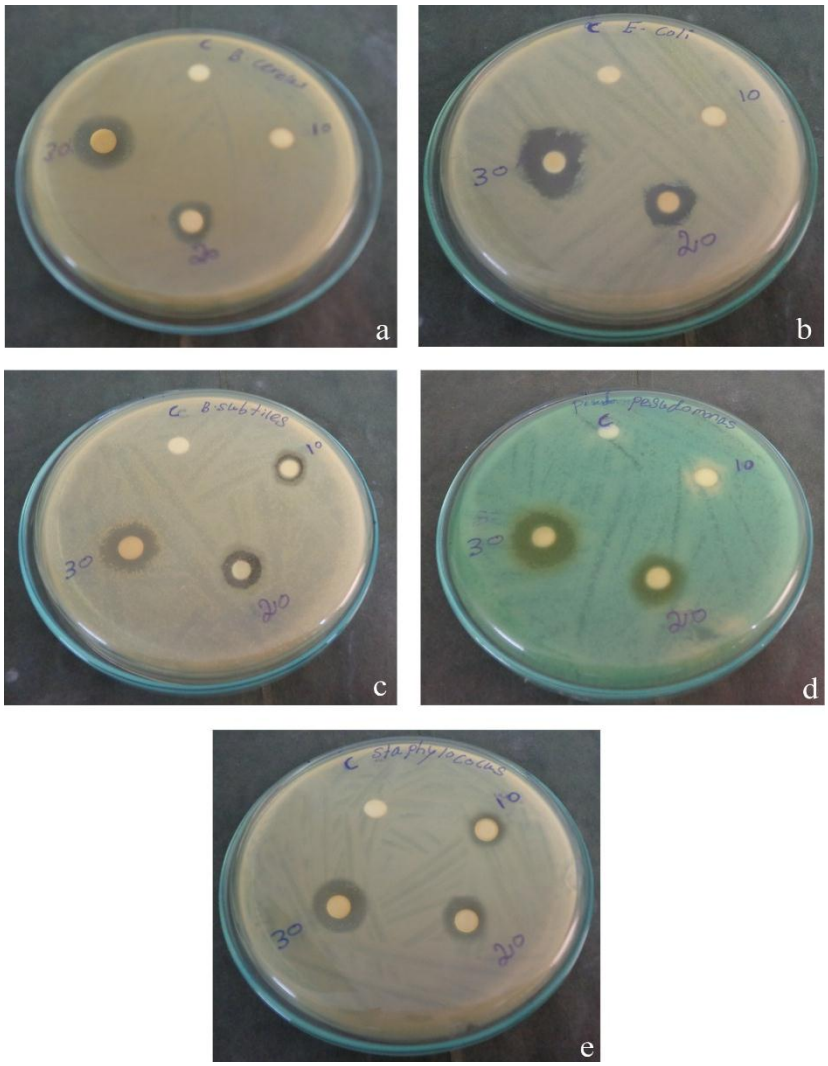

Figure 2: Antibacterial activity from ethanolic flower extracts of knock out rose.

Antibacterial activity of flower extract of knock out rose against (a) *Bacillus cereus*, (b) *Escherichia coli*, (c) *Bacillus subtilis*, (d) *Pseudomonas aeruginosa*, (e) *Staphylococcus aureus.*

REFERENCE

1. Roberts, AV., Horan, I., Metherws, D., Mottley, J. Protoplast technology and somatic embryogenesis in Rosa. In: de Jong J (ed) Proc. Eucarpia Symp. Integration of *In Vitro* Techniques in Ornamental Plant Breeding, CPBR, Wageningen, The Netherlands, 1990; 110-115.
2. Pati, PK., Rath, SP., Sharma, M., Sood, A., Ahuja, PS. *In vitro* propagation of rose: a review. *Biotechnology Advances*, 2006; 24: 94–114.

3. Ishioka, N and Tanimoto, S. Plant regeneration from Bulgarian rose callus. *Plant Cell Tiss. Org. Cult.* 1990; 22: 197–199.
4. Kornova, KM and Michailova, J. Study of the *in vitro* rooting of Kazanlak oil-bearing rose (*Rosa damascene* Mill.). *J. Essential Oil Res*., 1994; 6: 485-492.
5. Shimizu, T., Muroi, T., Ichi, T., Nakamura, M., Yoshihira, K. Analysis of red cabbage colors in commercial foods using high performance liquid chromatography with photodiode array detection-mass spectrophotometry. *J Food Hyg Soc Japan* 1997; 38(1): 34-38.
6. Faten, M., Hanen, F., Fellah, R and Chedly, A. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. *Journal of Taibah University for Science*. 2014; 8(3): 216-224.
7. Baskaran P and Jayabalan N. An efficient micropropagation system for *Eclipta alba* a valuable medicinal herb. *In vitro Cell Dev Bio,* 2005; 41: 532-539.
8. Gururaj, HB., Giridhar, P., Ravishankar, GA. Micropropagation of *Tinospora cordifolia* (wild) Miers ex Hook. F &Thoms - a multipurpose medicinal plant. *Current Science*, 2007; 92: 23-26.
9. Janarthanam, B and Seshadri S. *In vitro* manipulations of *Rosa bourboniana* Acta *Horticulturae*, 2008; 769: 357-370.
10. Janarthanam, B and Sumathi, E. *In vitro* plant regeneration from shoot tip explants of *Exacum travancoricum* Beedi. *Journal of Plant Tissue Culture & Biotechnology*, 2010; 20: 113-118.
11. Asano, G and Tanimoto, S. Plant Regeneration from Embryogenic Calli Derived from Immature Seeds in Milliature Rose Cultivar. 'Shortcake'; Somaclonal Variation, Cytological Study and RAPD Analysis. *Plant Biotechnalogy*, 2002; 19(4): 271 – 275.
12. Janarthanam, B and Sumathi, E. Phytochemical composition, tannin content, DPPH assay and antibacterial activity of peel extracts of *Punica granatum*. L, *World Journal of Pharmaceutical Researc*h. 2015; 4(11): 1895-1908.