

**GC-MS AND ANTIOXIDANT POTENTIAL OF METHANOLIC LEAF
EXTRACT OF *PUTRANJIVA ROXBURGHII* WALL.
(PUTRANJIVACEAE)**

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ABSTRACT

The genus *Putranjiva* of putranjivaceae, comprises of 18 species. The present investigation deals with the GC-MS and antioxidant study of methanolic leaf extract of *Putranjiva roxburghii* Wall. It plays a vital role in the traditional medicine system for fever, cold, rheumatism, infertility problem etc. GC-MS study showed presence of 11 bioactive compounds, among them D-Fructose, 3-O-methyl- is the major compound. The IC 50 value of methanolic leaf extract showed that it had effective antioxidant activity, so it could provide a significant bio-resource of antioxidants in food and pharmaceutical industry.

KEYWORDS: *Putranjiva roxburghii* Wall., GC-MS, Antioxidant activity, DPPH, Reducing Powder Assay, Total Antioxidant Assay,

Nitric Oxide Scavenging Assay, Chhattisgarh.

INTRODUCTION

Traditionally plants and their extracts are very popular for prevention and treatment of various diseases. Plants have bioactive phytochemical constituents that show certain physiological effect on human body. They form the base of modern drugs which is in use in today's world and lead to the development of innovative drugs.^[1] Information of chemical compounds play an important role in the discovery of therapeutic agent and with less side effects.^[2] To identify the bioactive constituents such as long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino, nitro compounds etc., GC-MS is an effective tool.

Hence, it is associated with particular detection techniques which have become a sophisticated means for analysis of various compounds.^[3]

Antioxidation process takes place in the presence of atmospheric oxygen or reactive oxygen species (ROS) and also used as stabilizer for polymeric products, petrochemical cosmetics, foodstuffs and pharmaceuticals. Antioxidants plays a vital role in the defense mechanism of the organism against pathogens and are able to delay or to inhibit the process of oxidation.

Free radicals generated during various biochemical reactions in the body, which creates oxidative stress, and that leads to an imbalance situation between the production of reactive species and antioxidant defense activity. Its implies several diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases.^[4] Several investigations are on for assessing the property of antioxidant from the natural products.^[5] Prevention and treatment of complex diseases like Alzheimer's and cancer by antioxidant based drugs have been reported during last three decades.^[6]

Secondary metabolites in plants including phenol, terpenes and various other plant extracts exert the action of antioxidant.^[7,8,9,10] Throughout the world plants are analysed for its medicinal properties, low toxicity and its economic values.^[11] It has been reported that diet rich with fresh fruits and vegetables reduce the chances of cardiovascular diseases and certain forms of cancer.^[12] Rasayana and Ayurveda the Indian traditional system of medicines have used several plants for the treatment of number of diseases.^[13]

The present study aims to report the presence of bioactive compounds by GC-MS technique and antioxidant property in the methanolic leaf extract of *Putranjiva roxburghii* Wall.

Collection and Authentication

Collection of plant material

The fresh plants of *Putranjiva roxburghii* Wall. were collected from Kunkuri, district Jashpur (Chhattisgarh, India) in the month of August 2015. Identification and authentication of the plant specimens were done by Dr. S. John Britto, The Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen has been deposited at the centre with accession no. RHT 67164 and RHT 67530. Plant parts were washed properly with deionised water to remove dust and dirt. The plant samples were air dried under shade at room temperature,

ground with electric grinder into fine powder and stored in air tight container for further use. The fresh samples were used for macroscopic and microscopic observations.



Putranjiva roxburghii Wall.

MATERIALS AND METHODS

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of these extracts was performed using a Perkin- Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm X 0.25 mm 1 D X 1 μ Mdf, composed of 100% Dimethyl polysiloxane). For GCMS detection, an electron ionization system with the ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 μ L was employed (split ratio of 10: 1); Injector temperature 250°C; Ion source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10 °C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas; software adapted to handle mass spectra, and chromatograms was a Turbo mass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight, and structure of the components of the test materials were ascertained.

DPPH Radical Scavenging Assay^[14]

The 3ml of DPPH (30mg/L) solution was added to 1ml of sample solution at different concentrations (10- 50µg/ml). The reaction mixture was mixed well and kept in the dark room for 30 minutes. The absorbance was measured at 517nm by using Lambda 35 UV/VIS Spectrometer. The absorbance of the samples was compared with that of the control and the standard (Ascorbic acid). The IC₅₀ value of samples (concentration of sample required to inhibit 50% of the DPPH free radical) was calculated using Log dose inhibition curve. The lower absorbance of the reaction mixture indicated higher free radical activity. The ability of the plant extracts to scavenge DPPH radical was calculated by the following formula:

DPPH scavenging effect (%) or Percent inhibition: = [(Abs Control – Abs Sample)] / (Abs Control)] x 100

Where, Abs control was the absorbance of the control (without extract) at 517 nm; Abs sample was the absorbance in the presence of the extract at 517 nm. The experiment was repeated in triplicate.

Total Antioxidant Capacity (Phosphomolybdenum Assay^[15]

The molybdate reagent was prepared by taking 1ml of 0.6M conc. H₂SO₄, 28mM sodium phosphate and 4mM ammonium molybdate in 20ml of distilled water, and final volume was made up to 50ml. The different concentrations of plant extracts ranging from 10µl to 50µl were treated with 2ml of distilled water and 2ml of molybdate reagent solution. The reaction mixtures were incubated at 95°C for 90 minutes in water bath. After incubation, the tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695nm by using Perkin Elmer Lambda 35 UV/VIS Spectrometer. Mean values of three replicates of the samples were calculated for each extract. Ascorbic acid was used as positive reference standard. The percent of antioxidant was calculated by the following formula:

Percentage of antioxidant (%): = [(Abs Sample – Abs Control) / (Abs Sample)] x 100

Where, Abs control was the absorbance of the control (without extract) at 695 nm; Abs sample was the absorbance in the presence of the extract at 695 nm. The experiment was repeated in triplicate.

Reducing Power Assay

Various concentrations of the plant extracts were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). This mixture was kept at 50°C in water bath for 20 minutes.

After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract and the contact time.

Scavenging of Nitric Oxide Radicals

Plant extracts were dissolved in DMSO for this quantification. Sodium nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) was incubated with different concentration (10-50µg/ml) of extracts and essential oil, tubes were incubated at 29°C for 3 hours. Control experiment was done without the test compounds but with equivalent amount of buffer in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagents. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with naphthylethylenediamine hydro-chloride was observed at 550 nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. The percentage inhibition was calculated and plotted in a graph and compared to standard.

$$\% \text{ inhibition} = [\text{O.D. of control} - \text{O.D. of Test} / \text{O.D. of control}] \times 100$$

Where, Abs control was the absorbance of the control (without extract) at 546 nm; Abs sample was the absorbance in the presence of the extract at 546 nm. The experiment was repeated in triplicate.

RESULTS AND DISCUSSION

Putranjiva roxburghii Wall. has been in wider use in the Ayurvedic and Unani systems. The leaves have significant use in gynaecological ailments. Besides they are used as components and for diseases like leukorrhea, infertility, menstrual problems, etc. Detailed pharmacognosy and pharmacological study revealed the presence of several active compounds responsible for their reported medicinal value. Gas Chromatography-Mass Spectrometry (GC-MS) profile includes 11 bioactive compounds: Azulene, D-Fructose, 3-O-methyl-, Myo-Inositol, 4-C-methyl-, n-Hexadecanoic acid, Methyl 7,11,14-eicosatrienoate, 1,1,1,3,5,5,5-Heptamethyltrisiloxane, Silicic acid, diethyl bis(trimethylsilyl) ester, 2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)-, 4-(4-Chlorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine,

Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl- and 4-Phenyl-3,4-dihydroisoquinoline. Among these detected compounds D-Fructose, 3-O-methyl- (62.69%) is the major compound showed highest value of peak area. (**Chart. 1 and Table No.1**).

Cellular damage caused by an imbalance between Reactive Oxygen Species (ROS), as Radical scavenging potential has been demonstrated on ulcer^[16], diabetes^[17], Alzheimer's disease, memory and cognitive function^[18], age related neurological dysfunction^[19], cardiovascular and renal disorders.^[20] Free radical scavenger related therapy may have the property to delay or prevent the disorders. Use of traditional medicinal plants provide an active strategy to prevent these disorders.^[21]

In the present investigation *P. roxburghii* Wall. had been selected on the basis of their traditional practises for various diseases. Methanolic leaf extract of the selected plant was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, reducing antioxidant power, total antioxidant assay and nitric oxide scavenging assay.

In the present study, the antioxidant capacity of *P. roxburghii* was evaluated using DPPH radical scavenging method by comparing with the standard ascorbic acid activity as a known antioxidant. In this experiment, the concentrations range from 10-50µg/mL and highest percentage of inhibition was 62.79% at 50 µg/mL. (**Figure 1**).

The total antioxidant assay of *P. roxburghii* was determined by phosphormolybdenum using standard Ascorbic acid. In phosphormolybdenum assay, the concentrations range from 10-50µg/mL it showed higher percentage of activity as 81.48% at 50µg/mL. (**Figure 2**).

The reducing power assay was found to be 88.78% at 50µg/mL in *P. roxburghii*. The concentration range from 10- 50µg/mL. (**Figure 3**).

Nitric Oxide (NO) scavenging assay is based on the scavenging ability of *P. roxburghii*, as well as ascorbic acid as standard. Maximum inhibition of NO was observed in the extracts of highest concentration (50µg/ml) for the samples. At this maximum concentration, inhibition was found to be 71.03% for ascorbic acid, which serves as the standard. (**Figure 4**).

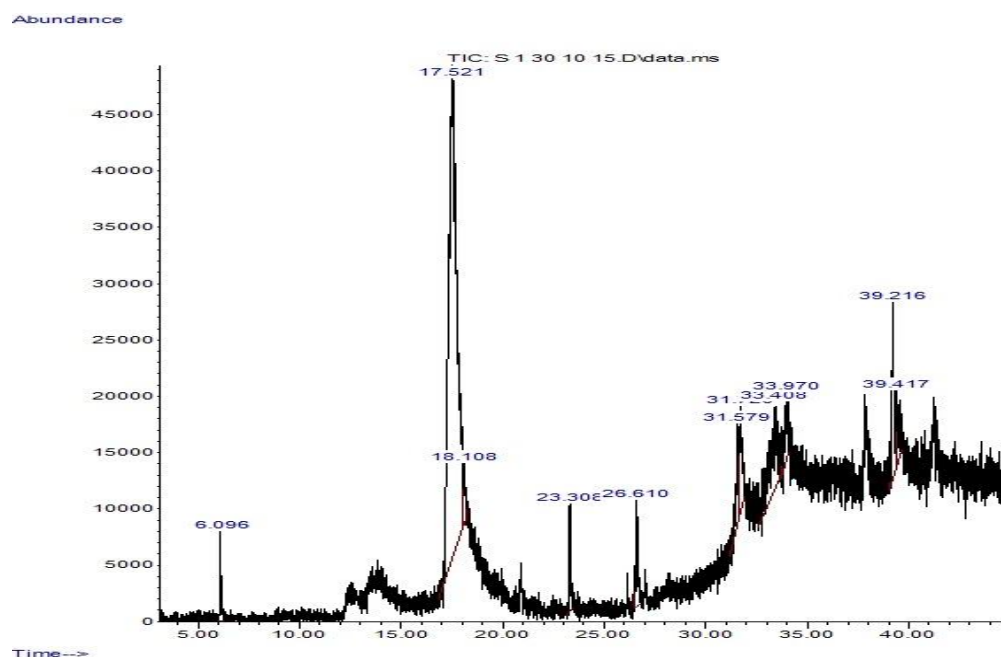
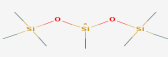
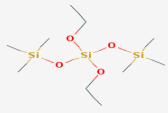
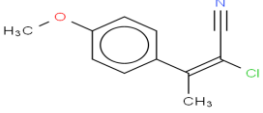
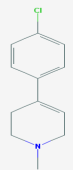
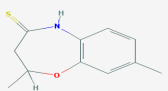
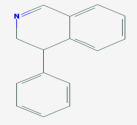


Chart-1: GC-MS Spectrum

Table No. 1: GC-MS analysis of Methanolic leaf extract of *P. roxburghii* Wall.

S.no	Name	Mol. Formula	Mol.wt.	Rt	Peak area	Structure
1	Azulene	C ₁₀ H ₈	128.17052 g/mol	6.097	1.56%	
2	D-Fructose, 3-O-methyl-	C ₇ H ₁₄ O ₆	194.18246 g/mol	17.523	62.69%	
3	Myo-Inositol, 4-C-methyl- / Laminitol	C ₇ H ₁₄ O ₆	194.18246 g/mol	18.107	1.43%	
4	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42408 g/mol	23.308	2.24%	
5	Methyl 7,11,14-eicosatrienoate	C ₂₁ H ₃₆ O ₂	320.50934 g/mol	26.610	3.07%	

6	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{21}O_2Si_3$	221.49694 g/mol	31.576	4.16%	
7	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296.58342 g/mol	31.720	3.81%	
8	2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)-	$C_{11}H_{10}$	207.6562g/mol	33.407	9.69%	
9	4-(4-Chlorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine	$C_{12}H_{14}ClN$	207.699 g/mol	33.968	2.63%	
10	Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl-	$C_{11}H_{13}NOS$	207.29202 g/mol	39.215	6.14%	
11	4-Phenyl-3,4-dihydroisoquinoline	$C_{15}H_{13}N$	207.27042 g/mol	39.416	2.57%	

Antioxidant activity of *P.roxburghii* Wall. LEAF SAMPLE

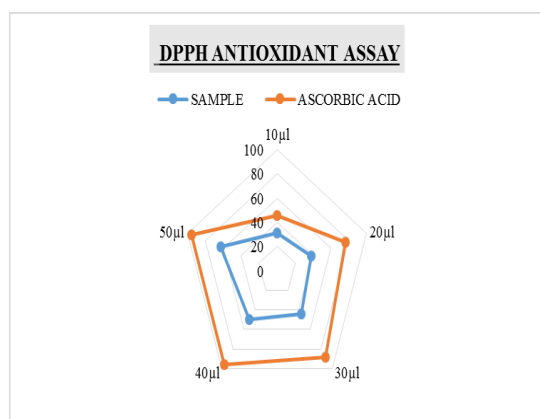


Fig.1: DPPH Scavenging assay of leaf extract of *Putranjiva roxburghii* Wall. compared to that of Ascorbic acid (Vit C). Each value is expressed as mean \pm standard deviation (n=3).

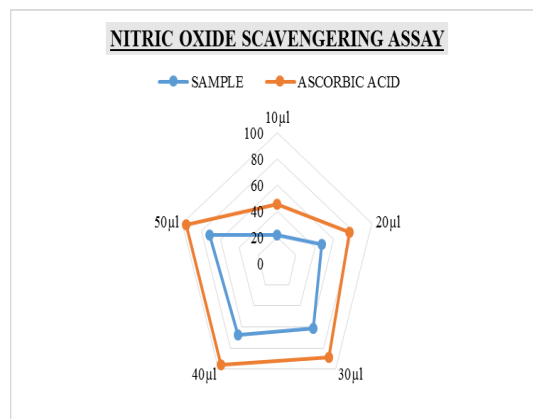


Fig.2: Nitric oxide scavenging of leaf extract of *Putranjiva roxburghii* Wall. compared to that of Ascorbic acid (Vit C). Each value is expressed as mean \pm standard deviation (n=3).

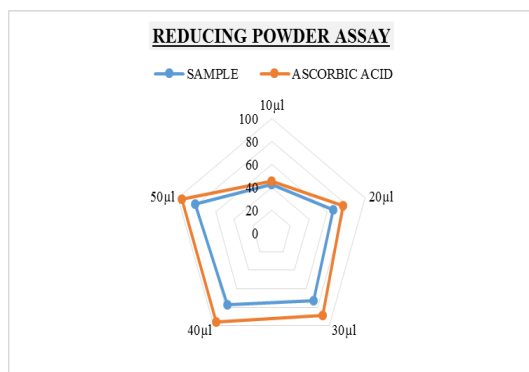


Fig.3: Reducing power assay of leaf extract of *Putranjiva roxburghii* Wall. compared to that of Ascorbic acid (Vit C). Each value is expressed as mean \pm standard deviation (n=3).

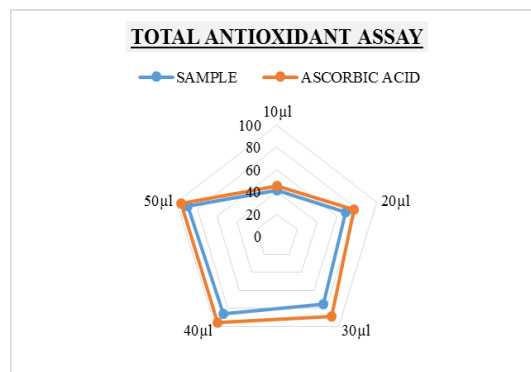


Fig.4: Total antioxidant assay of leaf extract of *Putranjiva roxburghii* Wall. compared to that of Ascorbic acid (Vit C). Each value is expressed as mean \pm standard deviation (n=3).

CONCLUSION

Using GC-MS analysis on the methanolic leaf extract of *Putranjiva roxburghii* Wall. bioactive phyto-compounds was prepared. The sample was subjected to specific tests of the antioxidant assay using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, reducing antioxidant power, total antioxidant assay and nitric oxide scavenging assay. The results have distinctly proved the antioxidant potential of *P. roxburghii* Wall.

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REFERENCE

1. Tagboto, S. and S. Townson, Antiparasitic properties of medicinal plants and other naturally occurring products. *Advances in Parasitology*, 2001; 50: 199-295.
2. Gupta, Y. and S. Briyal, Animal models of cerebral ischemia for evaluation of drugs. *Indian J Physiol Pharmacol*, 2004; 48(4): 379-394.
3. Kumar, B.S., Chemical Composition of Aqueous Leaf Extract of *Murraya koenigii*. *International Journal of Pharmaceutical & Biological Archive*, 2013; 4(3).
4. McCord, J.M., The evolution of free radicals and oxidative stress. *The American Journal of Medicine*, 108(8): 652-659.

5. Alam, M.N., N.J. Bristi, and M. Rafiquzzaman, Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 2013; 21(2): 143-152.
6. Ahmad, I., Z. Mehmood, and F. Mohammad, Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, 1998; 62(2): 183-193.
7. Yan-Chun, Z. and Z. Rong-Liang, Phenolic compounds and an analog as superoxide anion scavengers and anti oxidants. *Biochemical Pharmacology*, 1991; 42(6): 1177-1179.
8. Quinn, L.A. and H.H. Tang, Antioxidant properties of phenolic compounds in macadamia nuts. *Journal of the American Oil Chemists' Society*, 1996; 73(11): 1585-1588.
9. Seymour, T.A., S.J. Li, and M.T. Morrissey, Characterization of a Natural Antioxidant from Shrimp Shell Waste. *Journal of Agricultural and Food Chemistry*, 1996; 44(3): 682-685.
10. Prasad, K., et al., Evaluation of hydroxyl radical-scavenging property of garlic. *Molecular and Cellular Biochemistry*, 1996; 154(1): 55-63.
11. Auddy, B., et al., Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *Journal of Ethnopharmacology*, 2003; 84(2): 131-138.
12. Zahin, M., F. Aqil, and I. Ahmad, The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *International Journal of pharmacy and pharmaceutical Sciences*, 2009; 1(1): 88-95.
13. Chopra, R.N. and I. Chopra, *Indigenous drugs of India*, 1933. Academic publishers.
14. Mensor, L.L., et al., Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, 2001; 15(2): 127-130.
15. Prieto, P., M. Pineda, and M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, 1999; 269(2): 337-341.
16. Borrelli, F. and A.A. Izzo, The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy research*, 2000; 14(8): 581-591.
17. Sabu, M. and R. Kuttan, Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of ethnopharmacology*, 2002; 81(2): 155-160.
18. Howes, M.J.R., N.S. Perry, and P.J. Houghton, Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytotherapy Research*, 2003; 17(1): 1-18.
19. Delanty, N. and M.A. Dichter, Antioxidant therapy in neurologic disease. *Archives of neurology*, 2000; 57(9): 1265-1270.

20. Anderson, J.W., B.M. Smith, and C.S. Washnock, Cardiovascular and renal benefits of dry bean and soybean intake. *The American Journal of Clinical Nutrition*, 1999; 70(3): 464s-474s.
21. Shahwar, D. and M.A. Raza, Antioxidant potential of phenolic extracts of *Mimusops elengi*. *Asian Pacific journal of tropical biomedicine*, 2012; 2(7): 547-550.