Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Supple 1, 2015

Original Article

EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANT CAPACITY AND ANTIBACTERIAL ACTION OF THE FRUIT OF THESPESIA POPULNEA (L.) SOL. EX. CORREA

SELINA P SHAH[1*], ASHOK BHAGWAT[1]

[1]Shri C. B. Patel Research Centre, Vile Parle, Mumbai, India
Email: selinasns@gmail.com

Received: 10 Mar 2015 Revised and Accepted: 04 Aug 2015

ABSTRACT

Objectives: To carry out proximate analysis, detect phytochemicals, determine antioxidant activity, total polyphenolic content (TPC) and antimicrobial efficacy of the methanolic extract of the fruit of *Thespesia populnea* (T. populnea) and further carry out Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests for susceptible microorganisms.

Methods: The following methods were used: Folin Ciocalteau method for TPC, DPPH radical scavenging assay for *in vitro* antioxidant activity, agar well diffusion for antimicrobial activity and broth dilution for MIC followed by MBC.

Results: The following results were obtained: TPC of 78.12±6.46 mg/g GAE; IC_{50} value of the radical scavenging activity: 16.29 µg/ml; antibacterial activity-cold extract: excellent to moderate against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pasteurella multocida*, low to no effect against *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*; hot extract: excellent to moderate against *S. aureus*, *P. multocida* and *E. coli*; relatively low to no effect on *S. pyogenes*, *Candida albicans*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

MIC as well as MBC of the hot methanolic extracts of *T. populnea* were found to be between 0.1-2.5 mg/ml against *S. aureus*, *E. coli* and *P. multocida* whereas those of the cold extract against the same organisms was between 0.005-1.0 mg/ml.

Conclusion: The methanolic extract of *Thespesia populnea* fruit showed excellent antimicrobial activity against some dreaded pathogens besides being an antioxidant in nature. These properties of the fruit can help protect injured and/or infected tissues and hence be used to develop a novel medicinal agent.

Keywords: Proximate analysis, TPC, DPPH, Antimicrobial efficacy, *Thespesia populnea* (L.) Sol. ex. Correa.

INTRODUCTION

Tissue injury, such as seen during wounding, results in inflammation which in turn results in the release of cytokines and causes neutrophils and macrophages to produce free radicals [1]. If unregulated, the increase in pro-oxidants formed due to an increase in oxidative metabolism, result in a condition called oxidative stress. Prolonged exposure of free radicals, even at low concentrations, can lead to damage of biologically important molecules such as nucleic acids, proteins and lipids, and cause tissue injury and disease [2]. Antioxidants are substances that, when present in low concentrations compared to that of an oxidisable substrate, significantly delay, prevent or inhibit the oxidation of that substrate or remove the oxidative damage of the target molecule [3]. Intracellular antioxidant defenses such as catalase and superoxide dismutase, that are primarily enzymatic in nature, have evolved in order to protect the body from the ill effects of free radicals. A large amount of antioxidants such as transferrin, lactoferrin etc. extracellular. These antioxidants scavenge or suppress the formation of free radicals. During injury, an increase is seen in the production of reactive oxygen species (ROS), and this results in the consumption and depletion of endogenous scavenging compounds [4]. Hence, there is a need for additional antioxidants. Today, antioxidants are being increasingly used for protection against and for the repair of tissue damage. Synthetic antioxidants are also available, but none of the mimetics have received approval for clinical use [5]. Some researchers have reported synthetic antioxidants to be dangerous to health [6, 7]. Hence, the search for safe, nontoxic and natural antioxidants has been carried out by several researchers in the recent years [8-12].

Thespesia populnea (L.) Sol. ex. Correa, also known as the Indian tulip tree, Portia, False rosewood, Bhendi (Hindi) and Paraspipal (Marathi, Gujarati) belongs to the Malvaceae family. It is found throughout the coastal tropics of Africa, Asia and Europe [13]. This evergreen avenue tree is of medium size with distinctly cordate leaves and a yellow flower. The fruit consists of a somewhat flattened leathery sphere. The leaves, flowers, bark, fruits and seeds of this tree have been used for several ailments. The bark of this tree is mashed and used as a poultice or for hot fomentation in the treatment of wounds [14]. Traditionally, the bark and leaves of Portia are being used for production of oil used in the treatment of fracture wounds as well as for preparation of an anti-inflammatory poultice for application on boils and ulcers [15, 16].

The antibacterial, antiinflammatory, antidiabetic, and antioxidant activities of parts such as the leaves, bark and seeds of this tree have been studied by researchers [17-24]. However, similar effects of the fruit have not been extensively studied. The present investigation was carried out to screen the phytochemicals present in the fruit and study the *in vitro* antioxidant and antibacterial activity of the same in order to validate the ethnotherapeutic claims of its wound healing capacity.

MATERIALS AND METHODS

Collection, drying and extraction of the plant material

Fresh plant parts collected locally from the roadside trees in Santacruz, Mumbai were taxonomically identified by a botanist.

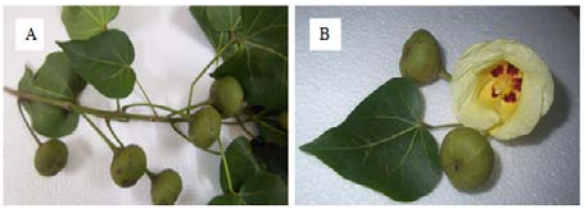

Fig. 1A: Leaves and fruit of *Thespesia populnea,* B: Leaf, flower and fruit of *Thespesia populnea*

The plant was submitted to the Blatter Herbarium, St. Xavier's College, Mumbai, and identified as *Thespesia populnea* (L.) Sol. ex Correa, family Malvaceae and found to match with the specimen no. NI 4821 of N. A. Irani. The fruit was shade-dried at room temperature for ten days. It was then ground into a powder and once again subjected to drying in an oven at 60 °C until completely dry. The powder was subjected to hot methanolic extraction (HME) by the Soxhlet's method. The dry powder was subjected to extraction by maceration at room temperature using methanol (cold, methanolic extract-CME). The extracts thus obtained (*T. populnea* fruit, hot–TpFH and cold–TpFC) were stored at room temperature (30 °C±2 °C) in amber coloured glass bottles till further use.

Proximate analysis

The physico-chemical analyses such as moisture content, ash content and alcohol soluble extractive value were carried out as per standard methods [25, 26]. All determinations were carried out in triplicate.

Phytochemical screening of the extract

The methanolic extract of the fruit of *T. populnea* was subjected to several tests for qualitative phytochemical analysis as per standard methods [27-29].

Total polyphenol content (TPC)

The total polyphenol content of the extract was estimated using the Folin Ciocalteau reagent based assay as previously described by Singleton and Rossi [30]. 25-400 µg/ml methanolic gallic acid solutions were used as standards and methanol was used as a blank. The absorbance of the developed colour was recorded at 765 nm using a UV-Vis spectrophotometer (Jasco V-550). All determinations, for gallic acid as well as the plant extract, were carried out in triplicate. Data are represented as an average of the three determinations. Using these readings, a calibrated gallic acid standard curve was made. Based on the measured absorbance of the plant extract, the concentration of phenolics was estimated (µg/ml) from the calibration line. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material).

Evaluation of antioxidant activity–*in vitro* method-free radical scavenging activity (DPPH)

Evaluation of antioxidant activity was done by an *in vitro* technique, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay adapted from Blois [31]. The method followed in this study was adapted from Afolayan *et al.* [32]. Various concentrations of ascorbic acid (0.4 to 6.0 µg/ml prepared in methanol) were used as standards. These were added to the freshly prepared DPPH solution (100 µM DPPH prepared in methanol); the mixture was rapidly vortexed and incubated in the dark at room temperature for 30 min. The absorbance of the ascorbic acid-DPPH mixture was read at 517 nm using a UV-VIS spectrophotometer (Jasco V-550) against a DPPH control containing methanol in place of ascorbic acid. Methanol was used as a blank. Using these readings, a calibrated ascorbic acid standard curve was made. Separately, various concentrations of the plant extract (5, 10, 20 and 40 µg/ml prepared in methanol) were added to freshly prepared DPPH solution (100µM DPPH in methanol). The tubes were rapidly vortexed and incubated in the dark at room temperature for 30 min. The absorbance of the test mixture was read at 517 nm using a UV-VIS spectrophotometer (Jasco V-550) against a DPPH control containing methanol in place of the extract. Methanol was used as a blank. All experiments were performed thrice and the results were averaged. The antioxidant capacities of various concentrations of the extract were determined based on the reduction in absorbance and expressed as percentage inhibition. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The IC_{50} value (µg/ml) was calculated and it denoted the concentration of extract required to scavenge 50 % of DPPH radicals [33].

Antibacterial assay

Microorganisms

The bacteria used for the assay included both Gram-positive and Gram-negative bacteria. A fungus was also included to test the antifungal activity of the extract. The Gram-positive bacteria used were *Staphylococcus aureus* MTCC 737, *Streptococcus pyogenes* (clinical isolate); the Gram-negative bacteria used were *Escherichia coli* MTCC 10148, *Klebsiella pneumoniae* (clinical isolate), *Pseudomonas aeruginosa* MTCC 424, *Pasteurella multocida* MTCC 1160; the fungal strain used was *Candida albicans* MTCC 227.

Inhibitory zone determination by agar well diffusion assay

The antimicrobial activity of the extract was determined according to the method described by Valgas *et al.* [34] with a slight modification. All nutrient media used in the experiment were obtained from Hi-Media Laboratories, Mumbai and prepared as per the manufacturer's instructions. Nutrient agar, Brain Heart Infusion (BHI) agar and Sabouraud's Dextrose agar slants were used to maintain cultures at 4 °C. The stock cultures were revived by inoculation in Mueller Hinton (MH)/BHI/Sabouraud's broth subsequently incubated for 18h at 37 °C. Their identity was ascertained morphologically and by biochemical tests. The inoculum was prepared from the broth by diluting with sterile physiological saline and standardized using 0.5 McFarland barium sulphate standard comparable to a bacterial suspension of 1.0×10^8CFU/ml. Each of the cultures was uniformly spread using sterile cotton swabs on separate plates containing sterile MH agar (*S. aureus*, *E. coli, K. pneumoniae* and *P. aeruginosa*), BHI agar (*S. pyogenes* and *P. multocida*) and Sabourauds dextrose agar (*C. albicans)* for determination of the antimicrobial activity. Wells were cut using a sterile cork borer of 8 mm diameter. Since methanol was used as a solvent for the plant extracts, one well was filled with methanol. The extracts were allowed to diffuse at room temperature (28-30 °C) for one hour. The plates were then incubated for 24h in an incubator maintained at 37 °C. Plates containing *C. albicans* were incubated for 48h. The antibacterial activity was determined by measuring the diameter of the zone of inhibition in millimeters (mm) in three different directions and a mean of triplicate results was taken. The experiment was repeated thrice and an average of the three values were recorded.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Mueller-Hinton broth cultures (24h old) were diluted with physiological saline (0.85 % w/v sodium chloride) to match a 0.5 McFarland standard to obtain inocula of approximately 10^8CFU/ml. After a preliminary, broad range MIC determination, serial dilution of the plant extracts was carried out using Mueller Hinton/Brain Heart infusion broth to obtain final concentrations between 0.001 and 2 mg per ml for TpFC and 0.05 and 3 mg per ml for TpFH. Each tube was inoculated with 100 µl of the bacterial suspension. The contents of the tubes were thoroughly mixed and incubated at 37 °C for 24h. After incubation, 50 µl of the broth was withdrawn from each tube and inoculated on agar plates which were incubated at 37 °C for 24h. The MIC value was determined from the broth media as the lowest concentration of the crude plant extract that inhibited the organisms, determined visibly by an absence of growth. The MBC value was determined as the lowest concentration of the crude plant extract that inhibited the organisms, determined by absence of growth on the plate.

RESULTS

Proximate analysis

The results of the quantitative parameters are shown in table 1. The moisture content of the dried and powdered fruit was found to be 14.4 %. The percentage of total ash and acid insoluble ash content was found to be 5.55 and 0.008 respectively. These values indicate the amount of organic and inorganic matter in the sample. The methanol extractive value was found to be 15.9 %.

Table 1: Proximate analysis of the dry, powdered fruit of *T. populnea*

Parameter	value±SD (%)
Moisture content	14.4±0.58
Total ash	5.55±0.48
Acid insoluble ash	0.008±0.002
Water insoluble ash	0.27±0.07
Alcohol extractive	15.9±1.66

Phytochemical screening

Preliminary phytochemical screening of the extract revealed the presence of phenolic compounds, proteins, alkaloids, steroids, carbohydrates, glycosides, flavonoids, tannins and saponins.

Total polyphenolic content (TPC)

The total polyphenolic content of the methanolic extract of *Thespesia populnea* fruit was determined by the Folin Ciocalteau reagent based assay and expressed as gallic acid equivalents (GAE). The regression equation of the calibration curve (y = 0.0089x-0.0868, r^2=0.9953) was used for the calculation. The phenolic content of T. *populnea* fruit extract was found to be 78.12±6.46 mg/g GAE.

Antioxidant activity

The DPPH assay was used to study the free radical scavenging activity of the plant extract. The results, as shown in table 2, were interpreted in terms of % inhibition.

Antimicrobial activity

The cold as well as hot methanolic extract of *T. populnea* exhibited notable antibacterial activity against *Staphylococcus aureus* (table 3). The cold extract was found to be more effective against the Gram positive organisms used *viz.*: *S. aureus* and *Streptococcus pyogenes* whereas the hot extract showed better antimicrobial activity against the gram negative organisms (*Escherichia coli* and *Pasteurella multocida*). Both extracts did not inhibit the growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and were scarcely effective in inhibiting the fungal strain used i.e.: *Candida albicans.*

Table 2: Antioxidant activity: free radical scavenging activity (DPPH) of *T. populnea* fruit extract

Conc. of plant extract (TpFH) (µg/ml)	% inhibition
5	6.14±0.46
10	17.84±0.60
20	39.76±1.43
40	66.55±0.21

The scavenging effect of crude extracts of *T. populnea* (fruit extract) was found to be 16.29 µg/ml (IC_{50} value). The % inhibition value was found to be 66.55 at the highest tested concentration (40 µg/ml). The results were compared with ascorbic acid as a standard (IC_{50}=3.06 µg/ml).

Table 3: Antimicrobial effect of hot and cold methanolic plant extracts of *T. populnea*

Test organisms	Diameter of zone of inhibition (mms)		
	Methanol (Control)	Plant Extract	
		TpFH (50 mg/ml)	TpFC (30 mg/ml)
Gram-positive bacteria:			
Staphylococcus aureus MTCC 737	NI	30.66±0.76	33.0±1.0
Streptococcus pyogenes (clinical isolate)	NI	12.0±0.5	18.0±1.0
Gram negative bacteria:			
Escherichia coli MTCC 10148	NI	17.0±1.32	11.66±0.29
Klebsiella pneumoniae (clinical isolate)	NI	NI	NI
Pseudomonas aeruginosa MTCC 424	NI	NI	NI
Pasteurella multocida MTCC 1160	NI	26.0±1.32	15.83±0.29
Fungal strain:			
Candida albicans MTCC 227	NI	12.0±0.5	12.0±0.5

Table 4: MIC and MBC values of the hot methanolic extract of *T. populnea*

Test organisms	MIC value (mg/ml)		MBC value (mg/ml)	
	TpFH	TpFC	TpFH	TpFC
Staphylococcus aureus MTCC 737	0.1	0.005	0.1	0.005
Escherichia coli MTCC 10148	1.0	1.0	1.0	1.0
Pasteurella multocida MTCC 1160	2.5	1.0	2.5	1.0

Determination of MIC and MBC

MIC and MBC tests were carried out for the three organisms listed in table 4. This study showed that *E. coli* and *P. multocida* has higher MIC and MBC values as compared to *S. aureus,* which means that a higher concentration of the extracts are required to inhibit their growth.

DISCUSSION

Several researchers have worked on various parts of *Thespesia populnea* and reported therapeutic properties of the same [17, 19, 35-40]. However, there are scarcely any reports on the possible medicinal use of the fruit of the tree. The wound healing properties of the fruit of *T. populnea* have been reported in only one paper [36]. However, the antioxidant and antimicrobial activity of the fruit extract have not been mentioned and the study involved the use of an aqueous extract of the fruit. Additionally, the fruit has been reported to have an antihyperglycemic and antihyperlipidemic effect [37, 41].

The aim of this study was to investigate the phytochemicals, phenolic content, antioxidant action and antimicrobial activity of the fruit and thereby validate folklore claims of the plant as an effective wound healing agent.

The relatively low moisture content is indicative that the keeping quality of the powdered sample is within acceptable range according to European Pharmacopoeia [42]. This indicates that the dry fruit powder can be stored for a long period without significant degradation by the growth of microbes or activation of enzymatic systems due to humidity. Tannins and flavonoids, found to be present in the extract have been earlier reported exhibiting wound healing activity [43].

Several plants reported to show various biological activities, including antioxidant activity, have been found to contain phenolic compounds. The methanolic extract of the fruit of *T. populnea* showed a good phenolic content. The antioxidant capacity of the phenolic compounds can be mainly attributed to their redox properties, which play an important role in neutralizing free radicals, decomposing peroxides, or quenching singlet and triplet oxygen [44]. The phenolic content of the extract used in this study correlates with the antioxidant activity. Hydroxyl groups of phenols enable them to scavenge radicals thus making them very important plant constituents [45]. Several researchers have noted a linear correlation between the total phenolic content and antioxidant activity [46-48].

The DPPH assay has been widely used to estimate the antioxidant capacity of plant extracts [48, 49]. When an antioxidant scavenges the free radicals by hydrogen donation (HAT–hydrogen atom transfer), the purple colour of the methanolic DPPH solution changes to a pale yellow and a characteristic absorbance is seen at 517 nms. In this study, the plant extracts were found to be an effective scavenger of DPPH radicals. This antioxidant action may be attributed to the presence of phenolic phytochemicals such as flavonoids and tannins that were found to be present in the extract. Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [50]. The role of antioxidants from plant extracts in wound healing has been widely researched and it has been found that free radical scavenging properties enhance wound healing [51]. Antioxidants have been found to play an important role in cutaneous tissue repair by significantly preventing tissue damage thereby stimulating the healing process [17, 52]. Some researchers studied the antioxidant activity of *T. populnea* and concluded that the activity was either due to stabilization of the cellular membrane or antiperoxidase activity [17]. Other researchers have suggested that the gossypol present in *T. populnea* may be responsible for exhaustion of neutrophils, which in turn may account for the anti-inflammatory property of the plant [53]. The antioxidant and anti-inflammatory properties of the plant, along with the antibacterial action observed in this study, may be strong contributing factors to the use of *T. populnea* as a therapeutic plan.

The antibacterial activity of a plant-based medicine is often considered significant in the prevention and management of a wound infection. Infections, particularly those of the mixed type, have been implicated in delayed or chronic wound repair [54]. In the present study, the antibacterial and antifungal activities of the methanolic fruit extracts of *T. populnea* were explored using the agar well diffusion method. This method has been found more sensitive and more suitable than the paper disc method, especially when any suspended particulate matter is likely to be present in the extract and interfere with the diffusion of the antimicrobial substance into the surrounding agar [34]. The activity of the extracts was assessed on the basis of the zone of inhibition. The MIC and MBC of the extracts were also studied. The results reveal that both the extracts are potential antibacterial agents against four of the six bacterial strains used in the study. Interestingly, both, the cold as well as the hot extract, showed a very large zone of inhibition against *S. aureus*, an organism that has been implicated in infections of the wound for several decades [55, 56]. The emergence of resistant strains and the dearth of effective antibiotics has led to a search for alternative antimicrobial agents. Dryden (2010) found *S. aureus* infections to be of primary clinical and epidemiological interest since strains of this microbe are commonly found in skin and soft tissue infections and have acquired an enhanced virulence and antimicrobial resistance. Researchers have emphasized the need for creativity in the development of therapeutic options [57]. The results of this study clearly indicate that the fruit of *T. populnea* offer great hope for one such therapeutic option against *S. aureus* (MIC–hot extract: 0.1 mg/ml, cold extract: 0.005 mg/ml). Severe *Pasteurella multocida* infections have been commonly encountered in bite wounds [58]. No vaccine is currently available against *Pasteurella* species. Chemotherapy for the treatment of *Pasteurella* infections has proved toxic for humans, is expensive, lengthy and often ineffective due to increasing antibiotic resistance [59]. Hence, effective antibacterial agents are required to treat infections caused by this organism. The fruit extracts of *T. populnea* presents a promise in this direction too. The hot methanolic extract of the fruit was found to effectively inhibit the organism and its MIC was found to be 2.5 mg/ml (hot extract) and 1.0 mg/ml (cold extract). The antioxidant and antibacterial action of *T. populnea* fruit extract may be attributed to individual phytoconstituents or a synergistic effect of some of them together and may help develop a less toxic and more effective wound healing agent. However, more work needs to be done in terms of isolation of the effective phyto chemicals, characterization and animal studies.

CONCLUSION

The results of this study indicate that *Thespesia populnea* fruit extracts exhibit a significant phenolic content and a promising radical scavenging effect of DPPH radicals in a concentration dependent manner. The antioxidant action of the plant is likely to afford the tissue protection against oxidative stress caused by free radicals which damage cells and vital biomolecules. Chain reactions triggered by free radicals are terminated by antioxidants by removing free radical intermediates [60]. Characterization of the active components of *T. populnea* responsible for radical scavenging action, is required. Carocho and Ferreira [61], in their review of antioxidants, concluded that although antioxidants have an impact on our health, the method of administration and quantity is debatable, especially since some antioxidants have been found to act as pro-oxidants under certain conditions and concentrations. Work is required in this direction.

The results indicate that the antioxidant and antibacterial potential of the extract that may be responsible for the ethnobotanical medicinal application of this plant. Thus, in addition to the leaves and bark, the fruit of this tree exhibits properties worthy of attention for development of an effective antioxidant and antimicrobial agent that may expedite wound healing.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

REFERENCES

1. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4:89–96.
2. Fang Y-Z, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872–9.
3. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia 2011;82:513–23.
4. Nijveldt RJ, Van Nood E, Van Hoorn DE, Boelens PG, Van Norren K, Van Leewen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001;74:418–25.
5. Wagener FADTG, Carels CE, Lundvig DMS. Targeting the redox balance in inflammatory skin conditions. Int J Mol Sci 2013;14:9126–67.
6. Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxy anisole in F344 rats. J Natl Cancer Inst 1983;70:343-7.
7. Kahl R. Synthetic antioxidants: biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. Toxicology 1984;33:185-228.
8. Gupta VK, Sharma SK. Plants as natural antioxidants. Nat Prod Radiance 2006;5:326–34.
9. Narayanaswamy N, Balakrishnan KP. Evaluation of some medicinal plants for their antioxidant properties. Int J PharmTech Res 2011;3:381–5.
10. Geethalakshmi R, Sakravarthi C, Kritika T, Arul Kirubakaran M, Sarada DV. Evaluation of antioxidant and wound healing potentials of *Sphaeranthus amaranthoides* Burm. f. Biomed Res Int 2013;1-7. doi.org/10.1155/2013/607109. [Article in Press]
11. Ngonda F. *In-vitro* anti-oxidant activity and free radical scavenging potential of roots of malawian *Trichodesma zeylanicumm* (burm. f.). Asian J Biomed Pharm Sci 2013;3:21–5.
12. Paudel B, Bhattarai HD, Kim IC, Lee H, Sofronov R, Ivanova L, *et al.* Estimation of antioxidant, antimicrobial activity and brine shrimp toxicity of plants collected from oymyakon region of the republic of sakha (Yakutia), Russia. Biol Res 2014;47:10.
13. Francis JK. *Thespesia populnea* (L.) Sol. ex Corrêa. In: Vozzo JA. editor. Tropical Tree Seed Manual. Reforestation; Nurseries and Genetics Resources; Part II, Species description. Washington DC; USDA Forest Service; 2002. p. 762-4.
14. Phanse MA, Patil MJ, Abbulu K. Review on pharmacological studies of *Thespesia populnea* linn. Int J Pharm Pharm Sci 2013;5:1–5.
15. Anonymous, Wealth of India. First supplementary Series, Vol-X, (Sp-w), Raw materials. Published by Niscom; 2003. p. 223-5.
16. Jayaweera DMA. Medicinal plants (indigenous and exotic) used in Ceylon. Part 4, National Science Council of Sri Lanka, Colombo; 1982.

17. Ilavarasan R, Vasudevan M, Anbazhagan S, Venkataraman S. Antioxidant activity of *Thespesia populnea* bark extracts against carbon tetra chloride–induced liver injury in rats. J Ethnopharmacol 2003;87:227-30.
18. Vasudevan M, Gunnam KK, Parle M. Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. J Ethnopharmacol 2007;109:264–70.
19. Parthasarathy R, Ilavarsan R, Karrunakaran CM. Antidiabetic activity of *Thespesia populnea* bark and leaf extract against streptozotocin induced diabetic rats. Int J PharmTech Res 2009;1:106-9.
20. Moon A, Khan A, Wadher B. Antibacterial potential of *Thespesia populnea* (Linn.) sol. ex corr. leaves and its corresponding callus against drug resistant isolates. Indian J Nat Prod Resour 2010;1:444-9.
21. Ganga Rao B, Umamaheswara Rao P, Sambasiva Rao E, Mallikarjuna Rao T, Praneeth D VS. Studies on phytochemical constituents, quantification of total phenolic, alkaloid content and *In-vitro* anti-oxidant activity of *Thespesia populnea* seeds. Free Radicals Antioxid 2011;1:56–61.
22. Sarma DS, Venkata A, Babu S, Krishna KR. Antioxidant and anti inflammatory activities of *T. populnea* linn, Int J Res Pharm Chem 2011;1:674-6.
23. Shekshavali T, Sivakumar Hugar. Antimicrobial activity of *Thespesia populnea* soland. Ex correa bark extracts. Indian J Nat Prod Resour 2012;3:128-30.
24. Krishnamoorthy S, Adaikala Raj G, Chandrasekaran M. Antibacterial and antifungal activity of leaves of *Thespesia populnea*. Int J Pharm Pharm Sci 2014;6:404–11.
25. Quality control methods for herbal materials. Updated edition of Quality control methods for medicinal plant materials WHO; 1998.
26. Gokhale SB, Kokate CK. Practical Pharmacognosy. 13th ed. India: Nirali Prakashan; 2009.
27. Trease GE, Evans WC. Pharmacology. 11th ed. London: Bailliere, Tindall Ltd; 1978. p. 60 - 75.
28. Khandelwal KR. Practical pharmacognosy-techniques and experiments. Pune, India: Nirali Prakashan; 1996.
29. Kokate CK. Practical Pharmacognosy. 4th Edition. New Delhi, Vallabh Prakashan; 2004. p. 123.
30. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
31. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 2002;26:1199-200.
32. Afolayan AJ, Jimoh FO, Sofidiya MO, Koduru S, Lewu FB. Medicinal potential of the root of *Arctotis arctotoides*. Pharm Biol 2007;45:486–93.
33. Adetutu A, Morgan W a, Corcoran O. Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extracts of *Bridelia ferruginea* leaf, a wound-healing plant of Nigeria. J Ethnopharmacol 2011;133:116–9.
34. Valgas C, de Souza SM De, Smânia EFA, Jr AS. Screening methods to determine antibacterial activity of natural products. Brazilian J Microbiol 2007;38:369–80.
35. Shirwaiikar A, Vasanth Kumar A, Krishnanand BR, Sreenivasan KK. Chemical investigation and anti-hepatotoxic activity of *Thespesia populnea*. Int J Pharmacogn 1995;33:305-10.
36. Nagappa AN, Cheriyan B. Wound healing activity of the aqueous extract of *Thespesia populnea* fruit. Fitoterapia 2001;72:503-6.
37. Satyanarayana T, Saritha T, Balaji M, Ramesh A, Boini MK. Antihyperglycemic and hypoglycemic effects of *Thepesia populnea* fruits in normal and alloxan induced diabetes in rabbits. Saudi Pharm J 2004;12:107-11.
38. Vasudevan M, Gunnam KK, Parle M. Antinoceciptive and anti-inflammatory effect of *hespesia. populnea* bark extract, J Ethnopharmacol 2007;109:264-70.
39. Saravanakumar A, Venkateshwaran K, Vanitha J, Ganesh M, Vasudevan M, Sivakumar T. Evaluation of antibacterial activity of phenol and flavonoid contents of *Thespesia populnea* flower extracts. Pak J Pharm Sci 2009;22:282-6.
40. Patil PH, Patil JY, Mahale JN, Patel JB, Surana SJ. Evaluation of antiulcer activity of the terpenoid fraction from the leaves of *Thespesia populnea* (L) (Malvaceae) in albino rats. Res J Pharm Biol Chem Sci 2010;1:495-513.
41. Belhekar SN, Chaudhari PD, Saryawanshi JS, Mali KK, Pandhare RB. Antidiabetic and antihyperlipidemic effect of *Thespesia populnea* fruit pulp extracts on alloxan-induced diabetic rats. Indian J Pharm Sci 2013;75:217–21.
42. Elena DL. Pharmacognostic methods for analysis of herbal drugs, according to European pharmacopoeia. In: Basnet P. editor. Promising Pharmaceuticals. In Tech; 2012.
43. Soni H, Singhai AK. A recent update of botanicals for wound healing activity. Int Res J Pharm 2012;3:1–7.
44. Osawa T. Novel natural antioxidants for utilization in food and biological systems. In: Uritani I, Garcia VV, Mendeoza EM. editors. Postharvest biochemistry of plant food-materials in the tropics. Tokyo, Japan: Japan Scientific Societies Press; 1994. p. 241-51.
45. Potterat O. Antioxidants and free radical scavengers of natural origin. Curr Org Chem 1997;1:415–40.
46. Chandel M, Sharma U, Kumar N, Singh B, Kaur S. Antioxidant activity and identification of bioactive compounds from leaves of *Anthocephalus cadamba* by ultra-performance liquid chromatography/electrospray ionization quadrupole time of flight mass spectrometry. Asian Pac J Trop Med 2012;5:977–85.
47. Sun J, Liu S, Zhang C, Yu L, Bi J, Zhu F, *et al.* Chemical composition and antioxidant activities of *Broussonetia papyrifera* fruits. PLoS One 2012;7:1–8.
48. Mekni M, Azez R, Tekaya M, Mechri B, Hammami M. Phenolic, non-phenolic compounds and antioxidant activity of pomegranate flower, leaf and bark extracts of four Tunisian cultivars. J Med Plants Res 2013;7:1100–7.
49. Egea I, Sánchez-Bel P, Romojaro F, Pretel MT. Six edible wild fruits as potential antioxidant additives or nutritional supplements. Plant Foods Hum Nutr 2010;65:121–9.
50. Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. BMC Complement Altern Med 2008;8:54.
51. Süntar I, Akkol EK, Nahar L, Sarker SD. Wound healing and antioxidant properties: do they coexist in plants? Free Radicals Antioxid 2012;2:1–7.
52. Barku VYA, Opoku-Boahen Y, Owusu-Ansah E, Mensah EF. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus*. Asian J Plant Sci Res 2013;3:69–74.
53. Benhaim P, Mathes S, Hunt T, Scheuenstuhl H, Benz C. Induction of neutrophil Mac-1 integrin expression and superoxide production by the medicinal plant extract gossypol. Inflammation 1994;18:443–58.
54. Bonham PA. Identifying and treating wound infection. J Gerontological Nursing 2009;35:12–6.
55. Howe CW. The problem of postoperative wound infections caused by *Staphylococcus aureus*. Ann Surg 1957;146:384–96.
56. Nakamura T, Daimon T, Mouri N, Masuda H, Sawa Y. *Staphylococcus aureus* and repeat bacteremia in febrile patients as early signs of sternal wound infection after cardiac surgery. J Cardiothoracic Surgery 2014;9:80.
57. Dryden MS. Complicated skin and soft tissue infection. J Antimicrob Chemother 2010;65 Suppl 3:35–44.
58. Chapple CR, Fraser AN. *Pasteurella multocida* wound infections—a commonly unrecognized problem in the casualty department. Injury 1986;17:410-1.
59. Ahmad TA, Rammah SS, Sheweita SA, Haroun M, El-Sayed LH. Development of immunization trials against *Pasteurella multocida*. Vaccine 2014;32:909–17.
60. Sies H. Oxidative Stress: Oxidants and antioxidants-(Review). Exp Physiol 1997;82:291-5.
61. Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol 2013;51:15–25.