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ORIGINAL ARTICLE

Extraction, characterization and biological studies of phytochemicals from *Mammea suriga*

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FT-IR analysis

Abstract The present work involves extraction of phytochemicals from the root bark of a well-known Indian traditional medicinal plant, viz. *Mammea suriga*, with various solvents and evaluation of their in vitro antimicrobial and antioxidant activities using standard methods. The phytochemical analysis indicates the presence of some interesting secondary metabolites like flavonoids, cardiac glycosides, alkaloids, saponins and tannins in the extracts. Also, the solvent extracts displayed promising antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Cryptococcus neoformans* with inhibition zone in a range of 20–33 mm. Further, results of their antioxidant screening revealed that aqueous extract (with IC_{50} values of 111.51 ± 1.03 and 31.05 ± 0.92 μg/mL in total reducing power assay and DPHH radical scavenging assay, respectively) and ethanolic extract (with IC_{50} values of 128.00 ± 1.01 and 33.25 ± 0.89 μg/mL in total reducing power assay and DPHH radical scavenging assay, respectively) were better antioxidants than standard ascorbic acid. Interestingly, FT-IR analysis of each extract established the presence of various biologically active functional groups in it.

1. Introduction

Nowadays, the use of natural formulations as medicine is gaining more popularity. In fact, several natural formulations which make use of herbal extracts have been found to be safer medicines with minimum side effects when compared to chemical drugs. According to the Bulletin of the World Health Organization (WHO), around 65% of the world's population relied on medicinal plants as their primary healthcare source [1]. Also, it has been estimated that about 50% of the medicines developed since 1980 have been natural products, their derivatives, or their analogs [2,3]. Further, it has been predicted that approximately 25% of the currently used modern medicines are derived from plants [3]. Amongst them, analgesics (morphine), cardiotonics (digoxin), anticancer drugs (paclitaxel and the vinca alkaloids) and the antimalarials (quinine and artemisinin) are noteworthy [4].

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Mammea suriga is a familiar endemic medicinal plant belonging to the family Clusiaceae, which grows abundantly in Western Ghats of India. The plant is well known for its diverse applications in folk medicine in our region. It is a large tree, growing to a height of 12–18 m and its bud is used as a minor spice. The flower buds possess mild stimulant, carminative and astringent properties and are used in the treatment of dyspepsia and haemorroid [5]. Its root-paste is widely used as medicine to cure partial headache [6]. The roots of *Mammea longifolia* (Wight) Planch and Triana are shown to contain interesting molecules, viz. coumarins surangin A, surangin B and taraxerol [7]. It was reported that the extract of stem bark of *M. longifolia* showed one more coumarin named surangin C [8]. Indeed, *Mammea* coumarins have been investigated to possess a wide array of biological activities. These coumarins are good radical scavengers [9] and are cytotoxic to human tumor cells [9–13] that suppress tumor growth in animal models [14] and also they exhibit anti-HIV [15], antifungal [16] and antibacterial [13,17] activities.

Encouraged by the reported medicinal properties of *M. suriga* genus and prompted by the usage of root bark extract of *M. suriga* in many traditional medicinal formulations of our region, it has been contemplated to concentrate our studies on phytochemical studies of this rarely explored species and to investigate its medicinal properties. Accordingly, in the present study, the phytochemicals root bark of *M. suriga* was extracted with various solvents and these extracts were studied for their in vitro antimicrobial and antioxidant properties. Their antimicrobial activities were determined using disc diffusion assay and the antioxidant activity was evaluated by reducing power assay and DPPH radical scavenging method. In addition, Fourier transform infra-red (FT-IR) spectral analysis of crude extract was carried out in order to predict the presence of various active functional groups which are responsible for their biological activities.

2. Methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), trichloro acetic acid and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, USA). Meullar Hinton agar media and fluconazole were purchased from HiMedia (Mumbai, India). Clairo mono cefoperazone sublactum was procured from Span Diagnostics Ltd. (Surat, India). Cefoxitin and ticarcilin clavulanic acid were purchased from Oxoid Ltd. (Basingstoke, UK).

2.2. Plant material

The root barks of *M. suriga* were collected from the Western Ghats of Karnataka, India, during winter season. Plant parts were packed immediately after picking and kept in cold (−20 °C) dark storage until processed. The plant specimen was identified with the help of an expert, Prof. P. D. Ramya Rai (Head, Department of Botany, Alva's College, Mangalore University, India). Voucher specimens (No. T 6789/2011) were prepared and deposited in the herbarium of Alva's College, Moodbidri, India.

2.3. Extracts preparation

The root bark of *M. suriga* was washed with water and shade dried at room temperature for 15 days. After drying, the sample was coarsely powdered with a grinder. The dry sample (60 g) was sequentially extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water (200 mL × 2, each solvent) for 14 h under constant agitation. The extracts were evaporated to dryness in vacuo and stored in cold until used. The extraction yield was expressed as

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the dry extract (g)}}{\text{Weight of the sample used for the extraction (g)}} \times 100$$

2.4. Preliminary phytochemical assay

Freshly prepared extracts were subjected to standard methods of phytochemical analyses [18,19] to detect the presence of phyto-constituents, viz. flavanoids, carbohydrates, glycosides, saponins, tannins, proteins and alkaloids.

2.5. Antibacterial screening

In vitro antibacterial screening of the plant extracts in DMF was carried out against five pathogenic strains, viz. *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Bacillus subtilis* (recultured) and *Serratia marcescens* (recultured) by the disk-diffusion method [20]. To standardize the inoculum density for a susceptibility test, $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard, was used. A 0.5 McFarland standard was prepared as described by Andrews et al. [21]. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. Sterile filter paper discs of 6 mm diameter were loaded with 20 µL of the plant extract dissolved in DMF (50 mg/mL) to yield a final concentration of 1000 µg/disc. The paper discs were allowed to evaporate and then placed aseptically on the surface of the inoculated agar plates. Standard clairo mono cefoperazone sublactum, cefoxitin and ticarcilin clavulanic acid were used as positive controls while DMF served as a negative control. The experiment was performed in triplicates under aseptic conditions. Plates were kept at 4 °C for 15 min for diffusion and then incubated for 18 h at 37 °C. At the end of the incubation period, the antibacterial activity was evaluated by measuring the inhibition zones. The mean value of the diameter of the inhibition zone of the triplicates sets was taken as the final value.

2.6. Antifungal screening

In vitro antifungal testing of the plant extracts in DMF was carried out against two pathogenic fungi *Candida albicans* (recultured) and *Cryptococcus neoformans* (recultured) by the disk-diffusion method on a potato dextrose agar plate. The Petri dishes were prepared in triplicates and maintained at 37 °C for 2–5 days. Flucanazole was used as the standard. The measurements were carried out as described in the antibacterial assay above.

2.7. Total reducing power assay

Total reducing power of plant extracts was evaluated as described by Barros et al. [22] with minor modifications. Ethyl acetate, ethanolic and aqueous extracts were selected for the analysis. However, petroleum ether and chloroform extracts were not selected because of their insolubility in the experimental solvent system. In the procedure, 1.0 mL of plant extract solutions (final concentration 20–1000 µg/mL) was mixed with sodium phosphate buffer (pH 6.6, 0.2 M, 2.5 mL) and