of transmission of infection in the community. To the best of our knowledge, molecular typing has not been used to trace the spread of pathogens in the currency. However, an attempt has been made in the present study to know the clonal relationship between the commonly isolated bacterial species using RAPD technique, though 50 samples tested represent a small fraction of the large number of coins and currency notes circulating in the community.

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Oily fraction of nuts of *Semecarpus anacardium* Linn. enhances iNOS expression in macrophages

Recently, we have reported that nonpolar oily fraction (NPF) and polar fraction of nuts of *Semecarpus anacardium* Linn. (Anacardiaceae; SA nuts) exhibit opposite property towards the pattern of release of nitric oxide (NO) by macrophages. NPF shows induction of NO production, similar to lipopolysaccharides (LPS), but the polar fraction has inhibitory effect against LPS-induced NO production[1]. Since no information is available about the role of NPF on the activity of iNOS enzyme or its gene expression, this aspect has been studied on rat peritoneal macrophage culture. The kernel of SA nuts is widely used in the Ayurveda and Siddha system of Indian medicine as anti-helminthic, antifungal, anti-carcinogenic, anti-nervous disorders and antiarthritis[2–7]. We have previously reported its antioxidant property[8] and anti-cancer property in DU-145 cells[4]. It also prevented localized bone loss and showed enhancement in calcification in osteoblast-like cells[9]. We have also developed a novel polyherbal formulation (BHUx patented in USA, EU, China and India), consisting of purified SA extract along with four other medicinal plants to manage hyperlipidemia and atherosclerosis, through its antioxidant and anti-inflammatory properties[10–11]. These properties were later associated with its active compound, tetrahydroamentoflavone, a biflavonoid. In Ayurveda, emphasis has been given towards its purification before clinical use[12].

LPS, phenylmethylsulphonyl fluoride, aprotinin, leupeptin, sodium dodecylsulphate and quercetin were purchased from Sigma, USA. Methelyne blue, ethylenediaminetetraacetic acid, NaCl, RPMI-1640, hydrogen peroxide and fetal calf serum (FCS) were purchased from Hi Media, Mumbai, India. Rabbit polyclonal anti-iNOS primary and secondary antibodies were purchased from Santa Cruz Biotechnology and other chemicals were of analytical grade.

Next, 250 g of dried SA nuts was carefully crushed and extracted in a soxhlet extractor with hexane for 30 h. The solvent-free dried hexane extract was standardized in terms of its percentage of yield, and TLC fingerprint was developed as described elsewhere[13]. Its stock solutions were made in dimethyl sulfoxide (DMSO) as 300 mg/ml and serially diluted with phosphate buffered saline (PBS) in three different working solutions (100, 10 and 1 mg/ml), which were used in culture wells in such a way that the final concentrations of NPF were arrived as given in the respective table, but the final concentration of DMSO was lower than 1%.

Animal experiments were approved by the Ethical Committee for Animal Welfare at the R&D Centre, Prof. S.N. Tripathi Memorial Foundation, Varanasi. Inbreed albino rats of Charles Faster strain were

Table 1. Effect of NPF on release of nitric acid (NO) in macrophage culture and iNOS protein expression

Treatment group	NO production (NO_2 μM/10^5 cells)	Percentage of cell viability by MTT assay (OD 540)	Pixel value	
			iNOS	β-actin
Normal cells	8.09 ± 1.987	2.211 ± 0.72	–	11,002
Control DMSO (0.23%)	10.76 ± 1.587	100 (2.198 ± 0.64)	–	10,987
SA-hexane fraction (NPF) (ng/ml)				
50	10.54 ± 1.539	99.89 (2.204 ± 0.61)	4667	11,054
200	20.76 ± 1.543*	99.98 (2.204 ± 0.45)	6794	10,968
400	42.89 ± 1.943*	99.54 (2.125 ± 0.87)	12,455	11,043
600	43.87 ± 1.749*	98.98 (2.003 ± 0.70)	14,796	10,978
EC_{50} (ng/ml)	163.66			
LPS (20 ng/ml)	43.08 ± 1.298*	98.56 (2.098 ± 0.78)	15,678	11,076

Values are significant when compared with control ($P^* < 0.001$).

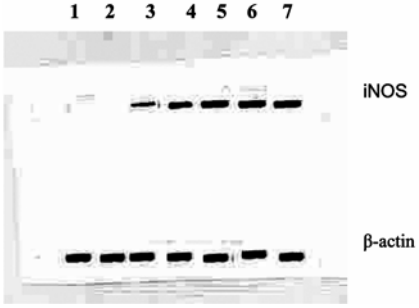

Figure 1. Effect of different concentrations of NPF on iNOS expression. Lane 1, Normal cells; lane 2, Control cells (treated with DMSO); lane 3, NPF (50 ng/ml); lane 4, NPF (200 ng/ml); lane 5, NPF (400 ng/ml); lane 6, NPF (600 ng/ml), and lane 7, LPS (20 ng/ml).

purchased from the Central Animal Facility of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. Isolation of macrophage and culture method were the same as described earlier[8]. It was cultured in complete RPMI-1640 medium, supplemented with 5% FCS.

All the tests were repeated three times in triplicate. Experimental control wells were treated with the same volume of diluted DMSO (drug vector), whereas in the extract-treated wells, different concentrations of SA hexane fraction (NPF) were added. After 17 h of incubation in CO_2 incubator maintained at 37°C, the released NO radicals were determined in culture supernatant. One set of cells was subjected to MTT assay[14] for assessment of viable cells and another set was subjected to Western blot analysis for assessment of iNOS protein expression. The concentration of NO released was equalized by the number of viable cells in each well.

The 20 μg protein of macrophage cells was separated using SDS–PAGE polyacrylamide gel[15] and the separated protein bands were transferred to nitrocellulose membrane by electro-blotting. The membranes were washed and incubated with rabbit polyclonal anti-iNOS antibody (SC650, Santa Cruz Biotechnology, diluted as 1/1000 in TBS-Tween-20 buffer) overnight at 4°C followed by secondary antibody, i.e. HRP-anti-rabbit IgG, where colour was developed using DAB (3,3-diamminobenzidine) as substrate. Equal loading was verified by monoclonal anti-β-actin antibody (Sigma) and band intensity was analysed using image analyser-2254, as described elsewhere[16]. All data were expressed as mean ± SD. Statistical comparison with control was done using SPSS 7.5 for Windows, SPSS Inc.

NPF was found to be highly toxic. The LC_{50} of NPF was found to be 50 μg/ml based on the regression curve drawn from the concentration response curve between 1 and 400 μg/ml final NPF concentration (data not shown). However, in a separate experiment, its LC_{50} was found to be 163.66 ng/ml and without any loss in cell viability up to 600 ng/ml. Thus this was used as the optimum concentration of NPF for all experiments with macrophage culture. Interestingly, NPF significantly enhanced the expression of iNOS protein (Figure 1) in a concentration-dependent manner along with proportional increase in NO release (Table 1).

The LC_{50} and EC_{50} values of NPF for NO release showed large difference. Therefore, the use of oily fraction of SA nuts could be exploited for therapeutic purposes, as described in Ayurveda[12]. Thus, increase in NO release accompanied by enhanced expression of iNOS proteins, suggests that NPF acts at the level of gene expression. Some reports indicate the anti-inflammatory property of SA oil, which could be because of its lower concentration, or may be due to partial contamination with the polar fractions, in the test preparation. However, its renal toxic effect has also been reported[17], which may be associated with its higher concentration, that would be responsible for cell damage. Thus, this study supports the necessity of purification of SA nuts to derive maximum anti-inflammatory property, and shows that the NO-releasing property of oily NPF fraction is mediated by enhanced expression of iNOS proteins.

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Variability in and relation between tree growth, heartwood and oil content in sandalwood (*Santalum album* L.)

Sandal (*Santalum album* L.) is traditionally and commercially popular for its heartwood and oil extracted from the heartwood (hereafter just 'oil'). Apart from many customary uses, the scented wood is suited for creating exquisite handicrafts, whereas the oil has well-known applications in perfumery and pharmaceutical industries[1]. There is a renewed interest for cultivation of sandal on private land due to the spiralling prices (an auction in Tamil Nadu in 2009–10 fetched a staggering Rs 53.27 lakhs for a tonne of sandalwood; Tamil Nadu Forest Department (pers. commun.)) and favourable amendments made to the rules governing sandal cultivation by the governments of Karnataka and Tamil Nadu[2], which account for nearly 90% of sandalwood available in the country[3]. Prior to these amendments, the rules declared it a royal tree, which credited every tree, including those grown in private land to be government property[4]. With green felling banned on forest lands in India and a widespread cry for forest conservation, it is imminent that private plantations are going to be the future source of sandalwood in the country. In this changing scenario, tree breeders and plantation managers may have to gear up for maximizing productivity of sandal on private lands; the hitherto practice of extracting naturally growing trees had not called for productivity maximization so far. The primary information required for the purpose will be: (1) quantified variation across individuals with respect to heartwood and oil content, and (2) relation between the two and the most easily measurable growth character, tree diameter. These will be crucial for developing efficient management schedules and for selecting superior performers.

There may be several reasons for the current paucity of data on these important traits. Crucial being the necessity for quantifying variation before the trees start maturing; maturity is known to culminate in a near-constant proportion of heartwood to the total tree diameter[5]. Therefore, it is best to assess variation in the heartwood content at the juvenile–mature interface, which in tropical hardwoods is approximately 20 years[6,7]. (The interface is characterized by having large variation in growth as well as other important wood traits such as density, strength and fibre length when compared with mature wood[8].) This puts information on age as an overriding necessity for quantifying variation, which has been lacking because heartwood is traditionally extracted from trees that naturally occur; known-aged plantations are not substantial even on forest lands. Additionally, surrogate measurements of age are hampered because of indistinct growth rings[9] and unreliability of girth approximations. In this context, the aim of the present study was to quantify variation in heartwood and oil content of sandal, and to work out their relationship with tree diameter among individuals at their juvenile–mature interface.

Trees used in the study were part of the germplasm bank of sandal established during 1982–83 by the Institute of Wood Science and Technology (IWST), Bangalore, which was then known as Sandal Research Centre. This bank was established at Gottipura Field Research Station, Hoskote (13°6′N, 77°48′E), which is approximately 35 km from Bangalore (unfortunately the entire germplasm was lost to thieves in 2003–2004). This germplasm was best suited for measuring variation, as it was an assemblage of sandalwood accessions from the existing natural populations in four southern states of India – Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. The assemblage was of those mature trees whose growth and heartwood content were considerably high at the time of selection. There was no information on their performance prior to maturity. Therefore, we still expected data from such an assemblage to reflect variation existing in sandal populations in arguably the centre of origin for the species. The study was conducted during 2002–2003 when the trees were 20 years old, which, we assume, is the approximate period when trees are at their juvenile–mature interface[6,7]. At the time of this study the germplasm consisted of 51 accessions, with each accession represented