Evaluation of *in vitro* antioxidant potential, anti-inflammatory activity and melanogenesis inhibition of *Artocarpus hirsutus* Lam. extracts

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Abstract: *Artocarpus hirsutus* Lam. belongs to Moraceae family and is endemic to Western Ghats and Kerala in India. This species is found to be effective in traditional medicine for the treatment of ulcer, diarrhea and pimples. However, extensive biological evaluation on each component of this specific species rarely appears in the literature which restricts its applicability as medicinal herb. The leaf, bark and wood of *Artocarpus hirsutus* Lam. were separately extracted with hot ethanol. The wood extract was further fractionated to isolate major active molecule whose structure was determined from its NMR spectra and LCMS analysis. All the extracts of *A. hirsutus* Lam. were then studied in vitro to evaluate their potential on tyrosinase inhibition, free radical scavenging activity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method and oxygen radical absorbance capacity (ORAC). Furthermore, their effects on melanogenesis inhibition were also evaluated by using murine melanoma cells. Activity guided fractionation of wood extract yielded a pure molecule that was characterized as oxyresveratrol. It was observed that antioxidant activity was higher in wood extract compared to the leaf and bark extracts. Isolated pure oxyresveratrol exhibited a significant antioxidant potential with ORAC value of 36,653±2570 μmol Trolox equivalent/g and having an IC_{50} of 4.3 μg/mL for DPPH free radical scavenging activity. This molecule was found to be effective for the tyrosinase inhibition with an IC_{50} of 0.1 μg/mL and melanogenesis inhibition in cultured melanoma cells by 44.62% at 0.2 μg/mL. Oxyresveratrol also exhibited significant inhibition of lipopolysaccharide (LPS) induced tumour necrosis factor alpha (TNF-α) secretion from J774A1 murine macrophage cell lines. This study provides substantial evidence for the presence of oxyresveratrol in the wood of *A. hirsutus* Lam. with promising anti-inflammatory, antioxidant and skin lightening property.

Key words: A. hirsutus Lam., melanogenesis, oxyresveratrol, TNF- α, tyrosinase.

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1. INTRODUCTION

Artocarpus hirsutus Lam. (Moraceae) is a perennial tree from the genus *Artocarpus*. It is endemic to Western Ghats of India and grows mainly in southern states of India [1], [2]. *Artocarpus* species such as *A. heterophyllus* (Jack fruit), *A. altilis* (bread fruit) and *A. hirsutus* (Monkey Jack) [3] produce edible fruits possessing good nutritional value and are in use as traditional medicine in India and South East Asian countries for the treatment of inflammation, malarial fever, ulcer, diarrhea, liver tonic, hypertension and diabetes. The pulp and seeds produced by *A. heterophyllus* are used as traditional herbal tonic; whereas roots of this species assist to control diarrhea and diabetes. The leaves of *A. lakoocha* protects against liver disorders, hypertension and diabetes [4]. Similarly, the rarely studied species of the genus, *A. hirsutus* Lam. [4], [5], [6], [7], [8], [9], [10], effectively relieves ulcers, diarrhea and soothing pimples [7]. It is also reported that the extracts of *Artocarpus* species have good cosmetic application [11]. Melanin pigments, produced by melanocytes, prevent skin damage apart from improving the skin colour. Abnormal hyper pigmentation causes melasma, post inflammatory melanoderma freckles and lentigines [12], [13], while hypopigmentation is associated with UV sensitivity and predisposition to skin cancer.

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Unusual activation of melanogenesis typically occurs due to repeated UV exposure, infectious/non-infectious biological factors, cutaneous pathological condition, or aging [14]. Cytokines / hormones / neuropeptides, including corticotrophin releasing hormone (CRH), proopiomelanocortin (POMC)-derived peptides (adrenocorticotropic hormone, α-melanocyte-stimulating hormone [α-MSH], β-endorphin), catecholamines, and acetylcholine via receptor-dependent/independent pathways are mainly involved in the hyper pigmentation [14], [15], [16], [17]. Oxidative stress and UV radiation leading to accumulation of free radicals play an important role in the growth of melanocytes. Free radical scavengers mostly reduce tyrosinase activity, thereby diminishing melanin synthesis, hyperpigmentation and melanogenesis [18]. Reactive oxygen species (ROS) induced oxidative stresses are potent inducers of NF-kB – the key regulator of inflammation. Thus, molecules possessing antioxidant activity often show remarkable anti-inflammatory property [19]. Skin whitening agents with anti-melanogenesis activity are used for curing hyper pigmentation and for the treatment of melanoma. In the last few decades, numerous tyrosinase inhibitors, including arbutin, kojic acid, hydroquinone and stilbenes have been marketed as skin whitening agents; however, their undesirable side effects necessitate the cosmetic and pharmaceutical industries to unearth novel skin whitening agents possessing potent efficacy without adverse effects for the benefit of human health [20]. Biological studies of *A. hirsutus* are limited to antimicrobial (root & fruit extract), antioxidant (fruit extract) and antiulcer (bark extract) activities [7], [10]; nevertheless, the antioxidant and the skin lightening potential of *A. hirsutus* extracts (from leaves, bark, and wood) are yet to bring the attention of the scientific community. The present study was thus designed to explore the antioxidant capacity and skin lightening potential of *A. hirsutus* Lam. extracts (from leaves, bark, and wood).

2. MATERIALS AND METHODS

2.1. CHEMICAL AND INSTRUMENTATION

The thin layer chromatography (TLC) on silica gel F_{254} plates (Merck Specialities Private Ltd., Mumbai, India) were visualized either under UV (254/366 nm) or by spraying with 5% vanillin-sulphuric acid reagent and heat for 10 minutes at 105 °C. ^{1}H and ^{13}C NMR spectra were measured on a Varian 300 MHz NMR Instrument. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) the reference standard. Coupling constants were presented in Hertz (Hz) and splitting patterns were presented as s - singlet; d - doublet; t - triplet; m - multiplet and bs - broad singlet. The purity of compounds was verified by analytical HPLC (Shimadzu Lab solutions). Mushroom tyrosinase, L-tyrosine disodium salt, Kojic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Fluorescien sodium salt were purchased from Sigma, India; Microplate reader was purchased from TECAN Ltd, Mannedorf, Switzerland; Flowstar OPTIMA reader was procured from BMG LabTech, Germany. The Spectrophotometer (UV-1650PC) was purchased from JASCO, Japan. DMSO, tert-Butylhydroquinone (TBHQ), 2,2'-Azobis-(2-amidonopropane) dihydrochloride and Trolox were purchased from Aldrich, India; 96 well black microtitre plate was purchased from Costar, Sigma-Aldrich, India. All reagents used were of analytical grade.

2.2. CELL LINES

The murine melanoma B16F1 cell line and murine macrophage cell line J774A1 were purchased from American Type Culture Collection (ATCC, Manassas, VA) and maintained as a monolayer culture in Dulbecco's Modified Eagle Medium (DMEM; Life technologies, CA, USA) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS; GIBCO/Invitrogen, Carlsbad, USA), 100 units/mL penicillin and 100 µg/mL streptomycin (Life technologies) at 37 °C in a humidified 5% CO_2 incubator.

2.3. PREPARATION OF THE EXTRACTS

Leaf, bark and wood of *A. hirsutus* Lam. was collected from Udupi district, Karnataka, India during January, 2014. All the samples were authenticated by botanist and sample voucher was preserved in herbarium (RD/HAR-AH/10, 11 and 12). The plant parts were cut into small pieces and dried in shade. The dried materials were pulverized to a coarse powder and stored in air tight containers. The powdered materials were separately extracted with soaked volume of hot ethanol. The ethanolic extracts were separated and dried completely. The extracts, leaf (LE), bark (BE) and wood (HWE) were stored at room temperature separately in air tight containers.

2.4. ISOLATION AND CHARACTERIZATION OF ACTIVE COMPOUND

The ethanolic extract from *A. hirsutus* Lam. wood (HWE) was redissolved in a small amount of ethanol and poured into water under stirring. Stirring was continued for 4 h and filtered to separate the water soluble (HWS) and water insoluble (HWI) fractions. The water soluble fraction was washed with equal volume of chloroform and discarded the chloroform layer. Then the water soluble layer was extracted with one volume of ethyl acetate and the ethyl acetate layer was dried completely to get the powder. The resultant powder was then poured into water and stirred at 80–90 °C for 8 h. After cooling at room temperature, the solution was filtered and the precipitated solid material was dried under vacuum at 70–75 °C. This isolated solid molecule was characterized as oxyresveratrol (OXY) from its ^{1}H and ^{13}C NMR spectra as well as from its LCMS spectrum and was comparable with the reported values. Purity of the compound was further verified by HPLC.

2.5. ASSESSMENT OF ANTIOXIDANT POTENTIAL

2.5.1.1 DPPH (1, 1-DIPHENYL-2-PICRYLHYDRAZYL RADICAL) SCAVENGING ASSAY

For the DPPH radical scavenging assay, 20 µL of extracts (LE, BE, HWE, HWI, HWS and OXY dissolved in DMSO) were mixed with 180 µL of DPPH in methanol (0.066 mM) in a 96 well plate following the method as described earlier [21]. The plate was kept in the dark for 30 min, after which the absorbance of the solution was measured at 516 nm using a microplate reader (TECAN Ltd, Männedorf, Switzerland). Blank (methanol), control (methanol and DMSO), standards (Trolox solution in DMSO) and test samples were recorded simultaneously. The extracts were screened with variable concentrations to establish the inhibition concentration (IC_{50}, the concentration reducing DPPH absorbance by 50%). The DPPH radical scavenging activity was calculated as follows,

$$\% \text{ Scavenging activity} = \frac{(T-T_c) - S}{S} \times 100 \tag{1}$$

Where,

T = Absorbance of Test solution+DPPH (in methanol)
T_c = Absorbance of Test solution+Methanol
S = Absorbance of DPPH+Methanol

2.5.1.2. OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC)

ORAC values of the extracts were estimated by following established method of Ou *et al.*, 2001 [22]. The reaction was carried out in 75 mM phosphate buffer (pH 7.4). *Artocarpus* extracts (25µL) and AAPH [2, 2'- Azobis (2-amidinopropane) dihydrochloride] solution (150 µL; 12 mM, final concentration) were mixed by using a multichannel pipette. Disodium fluorescein (150 µL; 9.6 X10^{-7}M, final concentration) solution was added rapidly and the microplate was immediately placed in the microplate reader to record the fluorescence at 485/520nm after every 1 minute upto 35 minutes (f_1……f_{35}). A blank (Fluorescein + AAPH) in phosphate buffer without antioxidant solution and five calibration solutions using Trolox (1-5 µM, final concentration) as antioxidant were also recorded for each assay. The final ORAC values are calculated by using a quadratic regression equation, ($Y = a + bX + cX^2$) between the Trolox concentration (Y) (µM) and the net area under the Fluorescence decay curve (X) and were expressed as micromoles of Trolox equivalents per liter or per gram of sample (µmol TE/L or µmol TE/g).

$$\text{The Area under curve (AUC)} = 1 + f_1/f_0 + f_2/f_0 + \ldots\ldots + f_{35}/f_0 \tag{2}$$

Where f_0 is the initial fluorescence reading at 0 minute and f_1 is the fluorescence reading after 1 minute. The data were analyzed by applying (2). The net AUC was obtained by subtracting the AUC of the blank from that of the sample. The value calculated using the net AUC of the sample and the quadratic regression equation was divided by the weight of the

sample in g/L. The final value obtained is the ORAC value of the sample expressed as µmol Trolox Equivalent/g.

2.6. CELL VIABILITY

Cells were seeded at a density of 5 x 10^3 cells/well in Dulbecco's Modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% antibiotics in 96 well plates and allowed to form a monolayer in a humidified incubator at 37 °C and 5% CO_2 atmosphere for 24 h. Cells were treated with different concentration of *A. hirsutus* Lam. extracts for 72 h to assess the cytotoxicity. Cell viability was determined by sulforhodamine B (SRB) assay [23]. Cells in DMEM with 0.1% DMSO was used as vehicle control. Absorbance at 570 nm was measured with microplate reader (TECAN Group Ltd., Männedorf, Switzerland). Cell viability was presented as the percentage of viable cells compared with untreated and control cells.

$$\% \text{ Viability} = 100 - \frac{\text{Absorbance (vehicle control)} - \text{Absorbance (sample)}}{\text{Absorbance (vehicle control)}} \times 100 \quad (3)$$

2.7. mushroom tyrosinase inhibition assay

Tyrosinase inhibition activity was determined using L-tyrosine as substrate in a 96 well plate [24]. A. hirsutus Lam. extracts were dissolved in DMSO. Each well contained 10 µL of sample with 140 µL of phosphate buffer (1.5 mM, pH 6.5), 10 µL of tyrosinase (40 units/mL), and 40 µL of L-tyrosine (3.53 mM). After incubation of the mixture at 37 °C for 10 min, absorbance was measured at 475 nm using a microplate reader (TECAN). For each sample a blank containing all components except enzyme was included. Kojic acid was used as positive control. The percentage of tyrosinase inhibition was calculated as follows:

$$\% \text{ Tyrosinase inhibition} = \frac{(Ec\text{-}B) - (T\text{-}Tc)}{(Ec\text{-}B)} \times 100 \quad (4)$$

Where,

Ec = Enzyme + Buffer + Substrate

B = Buffer + Substrate

T = Sample + Enzyme + Buffer + Substrate

Tc = Sample + Buffer + Substrate

2.8. MEASUREMENT OF CELLULAR MELANIN CONTENT

Cellular melanin content was determined as described previously [25]. In brief, B16F1 cells were seeded at a density of 5 × 10^4 cells/well in 24 well plates, treated with different concentrations of *A. hirsutus* Lam. extracts for 72 h, along with 0.5 nM of alpha-melanocyte stimulating hormone (α-MSH). Cells were scrapped off, peleted down and lysed using 2 N NaOH by keeping at 80 °C for 5 min. The absorbance of supernatant was measured at 405 nm using microplate reader to measure the melanin content.

2.9. ANTI-INFLAMMATORY ACTIVITY

J774 A1 cells were plated at a density of 1 x 10^5 cells/well in 96 well plates. Cells were treated with different concentrations of *A. hirsutus* Lam. extracts along with lipopolysaccharide (LPS) stimulation (0.1 µg/mL). The cell supernatants were collected 24 h after treatment and secreted TNF-α was estimated by cytokine ELISA kit (Boster Immunoleader, Pleasanton, CA, USA) as described by the manufacturer. Unstimulated cells served as negative control. The limit of detection was <1pg/mL.

2.10. STATISTICS

The experimental results were expressed as mean ± standard deviation (SD) of two independent experiments carried out in triplicates.

3. RESULTS

3.1. CHARACTERIZATION OF ACTIVE COMPOUND

The isolated pure molecule was characterized as 3,5,2',4'-tetrahydroxystilbene (Oxyresveratrol, OXY, Fig.1) from its analytical results that were similar to that of reported values [26], [27].

Fig. 1: The structure of oxyresveratrol
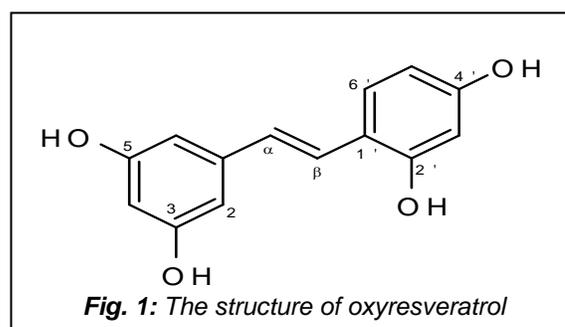
Oxyresveratrol: Pale brown fine powder with melting point 192-194 °C.
^{1}H NMR (DMSO-d_6 300 Hz) δ: 9.72 (1H, s, 5-OH), 9.54 (1H, s, OH), 9.32 (2H, s, OH), 7.33 (1H, d, J=8.7 Hz, H-6´), 7.14 (1H, d, J=16.5 Hz, H-α), 6.76 (1H, d, J=16.5 Hz, H-β), 6.34 (2H, d, J=2.1 Hz, H-2 and H-6), 6.30 (1H, d, J=2.4 Hz, H-3´), 6.24 (1H, dd, J=8.4 and 2.1 Hz, H-5´), 6.06 (1H, t, J=2.1 Hz,, H-4).
^{13}C NMR (DMSO-d_6 75 Hz) δ: 158.75 (C-3 and 5), 158.37 (C-4´), 156.30 (C-2´), 140.36 (C-1), 127.60 (C-6´), 125.00 (C-β), 123.60 (C-α), 115.61 (C-1´), 107.60 (C-5´), 104.34 (C-2 and 6), 102.87 (C-4), 101.70 (C-3´); APCI-MS m/z 245.00 (M+H$^+$) and 243.05 (M-H$^-$), ($C_{14}H_{12}O_4$ requires 244.2426).
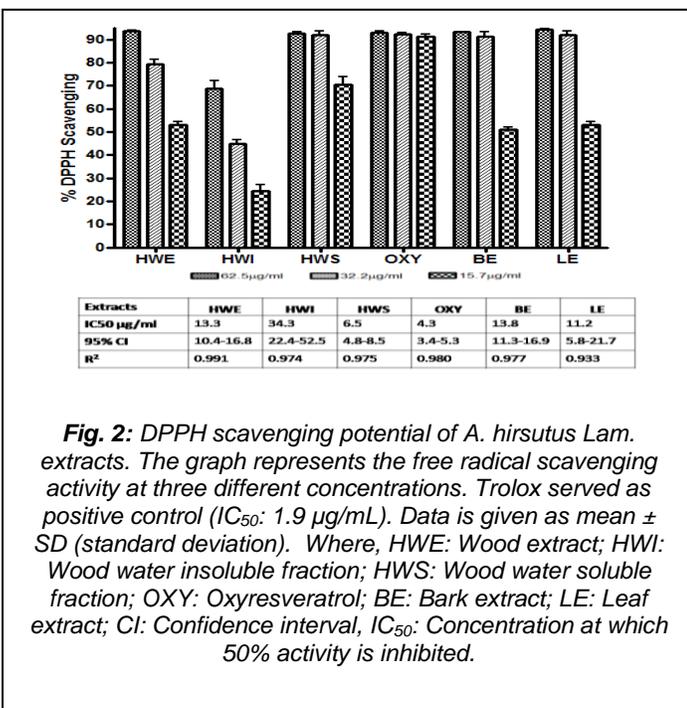
3.2. ANTIOXIDANT ACTIVITY USING DPPH (1, 1-DIPHENYL-2-PICRYLHYDRAZYL RADICAL) SCAVENGING ASSAY

DPPH, a stable radical, has been widely used to evaluate the free radical scavenging ability of various antioxidant actives [28]. The DPPH activity was estimated for leaf, bark and wood extracts of *A. hirsutus* Lam. and all the extracts exhibited significant DPPH scavenging activity with IC$_{50}$ of 11.25, 13.84 and 13.29 µg/mL respectively. The IC$_{50}$ of isolated pure oxyresveratrol was 4.3 µg/mL (Fig. 2). The order of the free radical scavenging capacity was observed as follows: OXY>HWS>LE>HWE>BE>HWI.

3.3. OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC)

ORAC assay measures the degree of inhibition of peroxy-radical induced oxidation by the compound [29]. The ORAC

activity was estimated for leaf, bark and wood extracts of *A. hirsutus* Lam. and the results are shown in Fig. 3. The ORAC value was significantly higher in the wood extract compared to leaf and bark extracts while, purified oxyresveratrol exhibited highest ORAC value 36,653±2570 µmol Trolox Equivalent/g as presented in Fig. 3.

Extracts	HWE	HWI	HWS	OXY	BE	LE
IC50 µg/ml	13.3	34.3	6.5	4.3	13.8	11.2
95% CI	10.4-16.8	22.4-52.5	4.8-8.5	3.4-5.3	11.3-16.9	5.8-21.7
R²	0.991	0.974	0.975	0.980	0.977	0.933

Fig. 2: *DPPH scavenging potential of A. hirsutus Lam. extracts. The graph represents the free radical scavenging activity at three different concentrations. Trolox served as positive control (IC_{50}: 1.9 µg/mL). Data is given as mean ± SD (standard deviation). Where, HWE: Wood extract; HWI: Wood water insoluble fraction; HWS: Wood water soluble fraction; OXY: Oxyresveratrol; BE: Bark extract; LE: Leaf extract; CI: Confidence interval, IC_{50}: Concentration at which 50% activity is inhibited.*
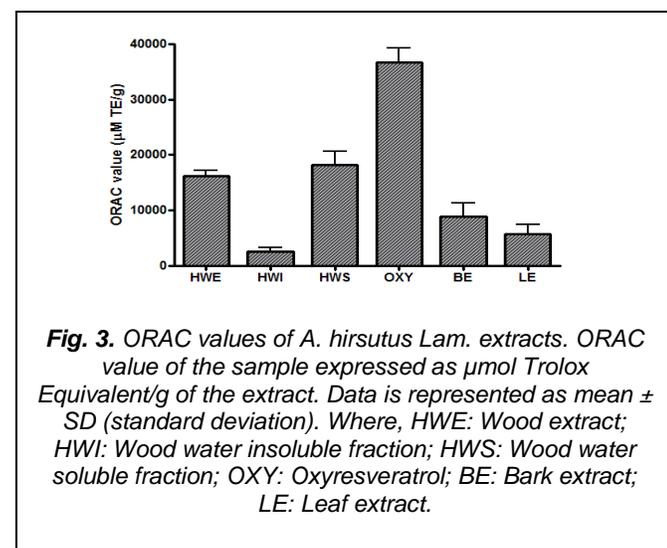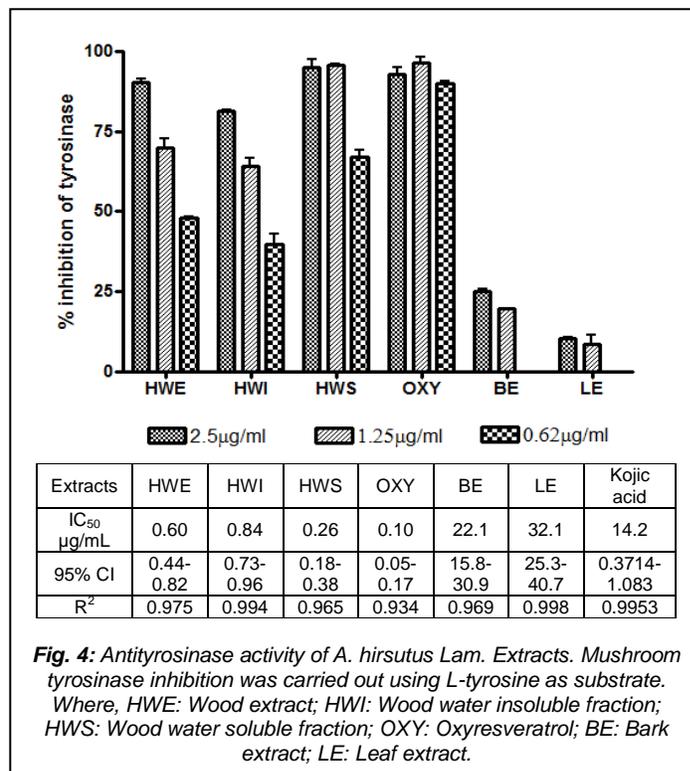

Fig. 3. *ORAC values of A. hirsutus Lam. extracts. ORAC value of the sample expressed as µmol Trolox Equivalent/g of the extract. Data is represented as mean ± SD (standard deviation). Where, HWE: Wood extract; HWI: Wood water insoluble fraction; HWS: Wood water soluble fraction; OXY: Oxyresveratrol; BE: Bark extract; LE: Leaf extract.*

3.4. TYROSINASE INHIBITION

3.4.1.1 INHIBITION OF TYROSINASE ENZYME ACTIVITY

The antityrosinase activity was estimated for leaf, bark and wood extracts of *A. hirsutus* Lam. Tyrosinase inhibitory activity was significantly higher in the wood extracts compared to leaf and bark extracts. The IC_{50} of purified oxyresveratrol was 0.10 µg/mL, which was 142 times more potent than kojic acid (IC_{50} = 14.2 µg/mL). The water soluble extract obtained from wood

also showed potent anti-tyrosinase activity with an IC_{50} of 0.26 µg/mL. The anti-tyrosinase activity of the bark and leaf extracts was found to be 53 and 36 times lower than the wood extracts. The tyrosinase inhibitions of various extracts of *A. hirsutus* Lam. are shown in Fig. 4. The order of tyrosinase inhibition was as follows: OXY>HWS>HWE>HWI>BE>LE.

Extracts	HWE	HWI	HWS	OXY	BE	LE	Kojic acid
IC_{50} µg/mL	0.60	0.84	0.26	0.10	22.1	32.1	14.2
95% CI	0.44-0.82	0.73-0.96	0.18-0.38	0.05-0.17	15.8-30.9	25.3-40.7	0.3714-1.083
R²	0.975	0.994	0.965	0.934	0.969	0.998	0.9953

Fig. 4: *Antityrosinase activity of A. hirsutus Lam. Extracts. Mushroom tyrosinase inhibition was carried out using L-tyrosine as substrate. Where, HWE: Wood extract; HWI: Wood water insoluble fraction; HWS: Wood water soluble fraction; OXY: Oxyresveratrol; BE: Bark extract; LE: Leaf extract.*

At a comparable concentration of 0.2 µg/mL, the soluble extract from wood showed 50.47% inhibition of melanin biosynthesis. Purified oxyresveratrol inhibited melanogenesis by 44.62% while the leaf extract showed an inhibition of 37.89%. Ethanolic extract of wood and the water insoluble fraction showed inhibition of 36.09% and 29.70% respectively at a higher concentration of 0.78 µg/mL, but no significant activity was observed in the bark extract. Kojic acid was used as positive control with 74.90% inhibition at 50 µg/mL. The IC_{50} of oxyresveratrol was 0.31 µg/mL, which is 83 fold higher compared to Kojic acid (IC_{50} of 25.75 µg/mL).

3.5. ANTI-INFLAMMATORY ACTIVITY

3.5.1.1. INHIBITION OF LIPOPOLYSACCHARIDE (LPS) INDUCED TNF-ALPHA RELEASE IN CULTURED MACROPHAGES

Inhibition of Lipopolysaccharide (LPS) (0.1µg/mL) induced TNF-α level in J774A1 cells was determined at different concentrations of *A. hirsutus* Lam. extracts. Significant reduction (60.30%) in TNF-α level was observed for the purified oxyresveratrol at 0.05 µg/mL. Anti-inflammatory activity was relatively low in the wood extract; however enhanced anti-inflammatory activity was observed in the water soluble fraction (Table.1). Significant anti-inflammatory activity was observed in the leaf and bark extracts.
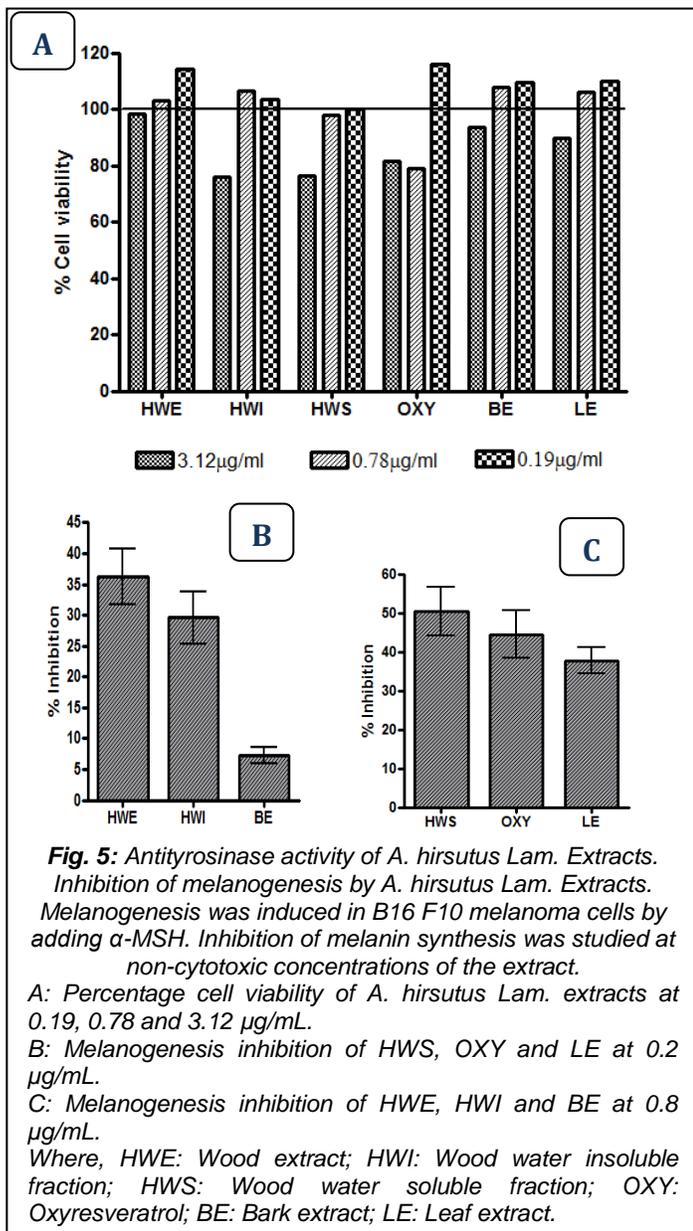
compared to kojic acid.

Fig. 5: *Antityrosinase activity of A. hirsutus Lam. Extracts. Inhibition of melanogenesis by A. hirsutus Lam. Extracts. Melanogenesis was induced in B16 F10 melanoma cells by adding α-MSH. Inhibition of melanin synthesis was studied at non-cytotoxic concentrations of the extract.*
A: Percentage cell viability of A. hirsutus Lam. extracts at 0.19, 0.78 and 3.12 µg/mL.
B: Melanogenesis inhibition of HWS, OXY and LE at 0.2 µg/mL.
C: Melanogenesis inhibition of HWE, HWI and BE at 0.8 µg/mL.
Where, HWE: Wood extract; HWI: Wood water insoluble fraction; HWS: Wood water soluble fraction; OXY: Oxyresveratrol; BE: Bark extract; LE: Leaf extract.

4. DISCUSSION

In the present study, we report the isolation and the biological activity of oxyresveratrol from the species *A. hirsutus* Lam. for the first time. The ethanolic extracts of leaf, bark and wood of *A. hirsutus* Lam. showed strong antioxidant and tyrosinase inhibitory activity in vitro. Although all the extracts significantly scavenged DPPH radicals with similar IC_{50} values, the ORAC value and tyrosinase inhibitory activity were much higher in wood extract compared to the leaf and bark extracts. To recognize the specific active molecule responsible for these activities, water was added into the ethanolic extract of wood to separate the water soluble and water insoluble portions. It was observed that the ORAC value of water soluble portion was notably higher than the insoluble portion. Oxyresveratrol was isolated from the water soluble portion, which showed excellent ORAC value, 36,653±2570 µmol Trolox Equivalent/g. The tyrosinase inhibitory activity of oxyresveratrol (IC_{50}) was found to be 0.1 µg/mL, which is 142 fold more potent than kojic acid, while melanogenesis inhibition was 83 fold higher

Table 1: Reduction in TNF-α secretion by A. hirsutus Lam. extracts in J774A1 cells.

A. hirsutus Lam. extracts	Concentration (µg/mL)	Concentration of TNF – α in the supernatant (pg/mL)	% inhibition of TNF–α secretion
LPS	0.1	7450	NA
HWE	1.5	6616	10.44
	0.75	7146	3.26
HWI	0.8	6857	7.17
	0.4	6909	6.47
HWS	0.4	2725	63.11
	0.2	2730	63.04
OXY	0.1	1346	63.56
	0.05	1465	60.32
BE	0.8	2575	65.14
	1.6	3188	56.84
LE	0.1	2321	68.58
	0.05	3238	56.17

Where, HWE: Wood extract; HWI: Wood water insoluble fraction; HWS: Wood water soluble fraction; OXY: Oxyresveratrol; BE: Bark extract; LE: Leaf extract.

The water soluble extract of wood also showed potent anti-tyrosinase and melanogenesis inhibition activity which can be explained by the presence of oxyresveratrol in the extract. Oxyresveratrol has been shown to possess a wide range of biological activity [30], [31]. It possesses potent anti-tyrosinase and skin whitening activity [32], [33], [34]. In addition, oxyresveratrol is a cyclooxygenase inhibitor, and possess anti-viral, anti-tumor and neuroprotective activities [35], [36], [37] [38]. Moreover, oxyresveratrol was reported to have lower cytotoxicity, better antioxidant activity and bioavailability than resveratrol, indicating its potential application as a food supplement, therapeutic and cosmeceutical agent [39], [40]. Oxyresveratrol has been isolated from several *Artocarpus* species (*A. lakoocha* Roxb.; *A. champlasha* Roxb.; *A. heterophyllus* Lam.; *A. gomezianus* Wall. [41], [42], [43], [44]. However there is no report of isolation of oxyresveratrol from *A. hirstutus* Lam. We have developed a simple, commercially viable unique process for the enrichment of oxyresveratrol. Previous reports on isolation and purification of oxyresveratrol engage time consuming chromatographic techniques involving preparative HPLC, column chromatography etc. [26], [27],

[42]. Whereas our method described herein to isolate good quality oxyresveratrol (purity >95% which is required for commercial purpose) was achieved based on solvent-solvent purification without any requirement of laborious chromatographic techniques. All the three extracts showed significant antioxidant and anti-inflammatory activity, among them, the wood extract was found to be the most active. Interestingly, the leaf and bark extract were not associated with melanogenesis inhibition or anti-tyrosinase activity, suggesting that the anti-inflammatory activity observed in these extracts could be due to the presence of other active molecules, which warrants further exploration.

5. CONCLUSION

Our study report describes an efficient method for isolation and purification process of oxyresveratrol from the wood of *A. hirsutus* Lam. without laborious, time consuming chromatographic techniques. The isolated compound was found to possess potent antioxidant, anti-inflammatory and melanogenesis inhibition activity comparable to the previous literature reports [34], [45], [46]. Thus the species *A. hirsutus* Lam. is a new alternate source for isolating oxyresveratrol, which may find application in skin lightening/whitening formulations. Further studies on other biological activity, bioavailability, stability and toxicity of formulation containing oxyresveratrol (in the form of cream, lotion, ointment etc) are recommended for its wider application in cosmetics.

Acknowledgment

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