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Analysis of chemical composition and assessment of biological potential of glowing compounds extracted from an exuviae of tasar silkworm *Antheraea mylitta*

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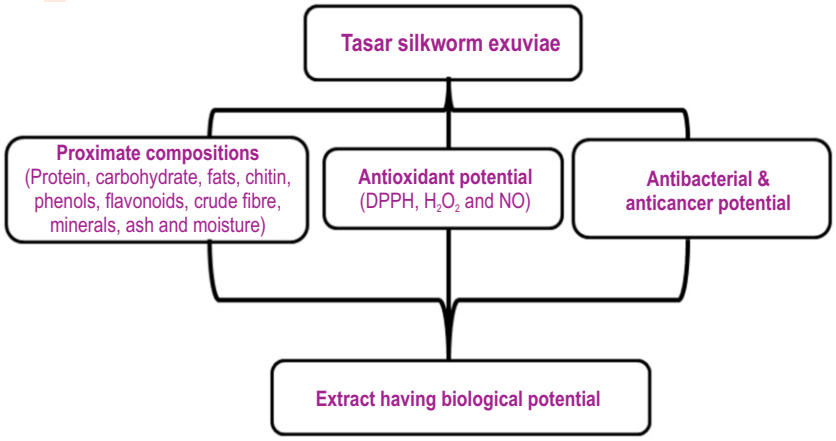
Abstract

Aim: Analysis of chemical composition and assessment of biological potential of tasar exuviae extracts.

Methodology: The biochemical components such as proteins, chitin, ash, fat, moisture, minerals and carbohydrate were estimated in the exuviae. After extraction, glowing pigments were characterized by UV-Vis and fluorescent spectra, and profiling of polyphenolic and flavonoids, free radical scavenging assay, antibacterial and anticancer potential were evaluated.

Results: The proximate components such as proteins, chitin, ash, fat, moisture, and carbohydrate contents were 62, 11.52, 10.57, 3.9, 3.1 and 0.9%, respectively. Further, among different elements, calcium level was higher than other elements such as magnesium, manganese, copper, zinc and sodium. The methanolic extract exhibited free radical scavenging and anti microbial potential in concentration dependent manner. In addition to these, glowing compounds prominent cytotoxic effect against breast cancer cell lines viz. MCF7, BT474 and SKBR3.



Interpretation: The action mechanism of extract could be probably due to the presence of yellow flavonoids (luteolin, catechin, epicatechin and quercetin) and phenolic acids (p-coumaric acid, gentisic acid and gallic acid), which glows during exposure of UV light and having biological potentials.

Key words: Anticancer, *Antheraea mylitta*, Tasar exuviae

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Introduction

During normal cellular functions in living organisms, there is endogenous production of reactive oxygen species (ROS) which are also exogenously produced through exposure to many xenobiotic substances (Halliwell and Gutteridge, 2001). In cellular systems, the levels of ROS are physiologically balanced at tolerable concentrations by both enzymatic and non-enzymatic antioxidants, and the imbalance leads to disruption of cellular functions (Halliwell and Gutteridge, 2001; Maxwell 1995), which results in several human diseases, like arthrosclerosis (Steinberg *et al.,* 1989), cancer (Muramatsu *et al.,* 1995), gastric ulcer (Das *et al.,* 1997), aging, etc. (Aruoma, 1994). In recent times, there is global attention to search for natural antioxidants which can be used in foods or as medicines to substitute artificial or synthetic antioxidants, which are otherwise harmful to human beings leading to manifestation of different diseases (Ito *et al.,* 1983; Zheng and Wang, 2001). Thus, the research on natural antioxidants has paramount importance. In this context, extract having proximate composition of phenolics and flavonoids are of great interest, due to their high potency of antioxidants and free radical scavengers, because of the redox nature, allowing them to function as donor of hydrogen, agent in reduction reaction, scavenger of singlet oxygen species and stabilize the phenoxyl radical (Rice-Evans *et al.,* 1996; Ramarathnam *et al.,* 1997; Wojdylo *et al.,* 2007).

India's sericulture comprises of both mulberry (*Bombyx mori*) and vanya silkworms *viz.* eri (*Samia ricini*), tropical tasar (*Antheraea mylitta*), muga (*A. assamensis*) and temperate tasar (*A. proylei*), which are commercially exploited for silk production in India (Gangopadhyay, 2008). In last decade, Indian tasar silk industry has indexed a remarkable growth in tasar raw silk production from 1166 MT in 2010-11 to 2689 MT in 2020-21 (Annual Report, Central Silk Board, 2020-21). Further, increased raw silk production is also associated with the generation of various industrial byproducts such as pupae, moths, exuviae, waste fibers etc., during the value chain of silk production, which causes disposal problems. Besides cocoon selling, it is highly essential to find out alternative sources of income by the multiple uses of non-silk by-products. There are few literatures available on the chemical composition and its medicinal (free radical scavenging) properties of exuviae (the discarded exoskeleton part of silkworm), a tasar silk waste by-product. It is estimated that an astounding 314MT of exuviae is being generated from 3136 MT of raw silk which is thrown away as waste. This industrial byproduct can be a valuable resource of pharmaceutical, cosmeceutical and allied areas. Thus, the present study was undertaken to assess the chemical composition and glowing antioxidant properties of this silk industrial by-product.

Materials and Methods

Collection of tasar silkworm exuviae: Tasar silkworms were reared on economic plantations of *Terminalia arjuna* during the month of September to November 2021 at Central Tasar Research and Training Institute, Ranchi field laboratory. As the growth of silkworm progress, the larvae cast off the exoskeleton. After completion of fifth and final instar, it spins cocoon and larva gets transformed into pupa, thus casting off the exuviae inside the cocoon. The exuviae were collected from the cocoons, and stored for further analysis.

Extraction of glowing antioxidants: The exuviae were washed properly using double distilled water and subjected to drying at 55°C for 24hr in a hot air oven. Thereafter, exuviae were crushed thoroughly, and immersed in 90% methanol (1:10 w/v) in an air tight container with stirring interval of 2-3 hr. After 72 hr the filtrate was collected by passing through Whatman's filter paper. The filtrate was evaporated and the powder thus obtained was stored at 4°C for further analyses.

Chemical composition of exuviae: Moisture, ash and fat contents were assayed by following the protocols of Association of the Official Analytical Chemists (AOAC, 2006). The total protein content was estimated by multiplying the factor 6.25 with the total nitrogen content assessed through Kjeldahl procedure. The quantitative estimation of carbohydrate was done by using anthrone method as described by Dubois *et al.* (1956) where D-glucose was used as standard. The total phenolics were estimated by Folin Ciocalteau Reagent (FCR) (Thimmaiah, 2006). Elements (Ca, Mg, Mn, Cu, Na and Zn) were analyzed through ICP-OES (Avio 200, Perkin Elmer, USA) at Orissa University of Agriculture and Technology, Bhubaneswar.

Profiling of phenolic acids and flavonoids by LCMS: Phenolic acids and Flavonoids profiling were analyzed through LC-MS/MS (Waters Acquity UPLCH class system fitted with TQDMS/MS system) at Indian Institute of Horticulture Research, Bangalore.

DPPH scavenging assay: DPPH is known to be a stable free radical compound. The basis of assay was to measure the reduction of DPPH by addition of exuviae which was detected by low intensity color formation. DPPH assay was conducted by following the protocol of Blois (1958) with minor modification. DPPH-methanol solution (1 ml, 0.1 mM solution) was mixed properly with 50-200 µg of exuviae extract. Absorbance of the solutions was recorded by spectrophotometer at 517 nm before and after incubation for 60 min at 37°C. For positive control butylated hydroxytoluene was used. DPPH scavenging was expressed as percentage using formula: $[A_{control-} A_{sample}/A_{control}] \times 100$ ($A_{control}$ = absorbance without extract and A_{sample} = absorbance with sample extract with different concentrations).

H_2O_2 scavenging assay: Among the reactive oxygen species, hydrogen peroxide was prominent due to generation of $OH^{\bullet}$ radical. The potential of exuviae for scavenging free radicals was conducted by the method of Mukhopadhyay *et al.* (2016). Hydrogen peroxide was added to the exuviae extract at different doses (0.3 mg and 1.2 mg) and the mixture was kept at room temperature (25°C) for 30 min. After the completion of incubation, ammonium ferrous sulphate (0.25 ml, 1mM) was added followed by addition of 1,10-phenathroline. The reaction mixture

developed red colour and after 30 min absorbance was recorded at 510 nm using UV-vis spectrophotometer. Ascorbic acid was used as positive control. The scavenging was calculated as percentage by using the formula: (A_{test}/ A_{blank}) x 100, (A_{test} = absorbance of test sample containing H_2O_2, and Ablank = absorbance of blank sample containing Ammonium ferrous sulphate and 1,10-phenathroline).

Assay for nitric oxide scavenging: Nitric oxide scavenging potential was assayed using spectrophotometric method (Sreejayan and Rao, 1997). Extract was prepared with methanol and added to series of test tubes in different concentrations (0.3, 0.6, 1.2 mg ml^{-1}). After addition of sodium nitroprusside, the mixture was incubated for 30 min at 25°C, followed by addition of 1.5 ml of Griess reagent which contained 1% sulphanilamide, 0.1% naphthyl ethylene-diamine dichloride and 3% phosphoric acid. The absorbance of test samples was recorded at 546 nm, and compared with that of nitrite solution as standard. Ascorbic acid was taken as standard solution and the buffer was used as blank. The percentage inhibition was determined by using the formula: [($A_{test\ or\ standard}$)/ $A_{control}$] x 100, where $A_{test\ or\ standard}$ is the absorbance of taste (ME) or standard sample (ASA), and $A_{control}$ is the absorbance of control samples (Sodium nitrite and Griess reagent).

Antibacterial activity: Fluorescent pigments were dissolved in methanol and were subjected to filtration on Millipore filters for sterilization. For estimating the antimicrobial activity of the pigments against *Bacillus subtilis*, *Candida albicans*, *Enterococcus farcalis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa* and *Streptococcus mutansdisc* diffusion method was adopted. Filter paper disks (5 mm radius) were soaked with 5-25 µl of each extract (50-250 µg/disc) and kept on the inoculated Petri dishes. Methanol solvent was used as negative control and tetracycline was used as positive controls. Petri dishes were incubated for 24 hr at 37°C. The inhibition zone against the studied microorganisms including disc diameter was measured which depicted the antimicrobial activity.

Cell viability assay: The effects of methanolic extract on cellular proliferation and viability were determined using the WST-1 assay. All cells (MCF7, BT474 & SKBR3) were seeded in 96-well ELISA plates at a density of 0.007x 10^6 cells per well (7000 cell per well) in 100µl of medium and allowed to attach to the surface overnight. On the following day, culture media was taken out and replaced with fresh one. Thereafter the cells were treated with methanolic extract at increasing concentrations (0.1, 0.5, 1, 2 mg ml^{-1}) along with methanol control and only cells as control for 24, 48, and 72 hrs. Subsequently, cells were incubated with the WST-1 reagent ($1/10^{th}$ of media volume/well) for 2 hr. The absorbance was recorded (450 nm and 620 nm) using an enzyme-linked immunosorbent assay plate reader (Multiscan EX, Thermo Scientific). Decreased cell viability in different exposure groups were expressed as the percentage of viable cells in the treated cells compared to the control.

Statistical analyses: All the experiments were conducted in six replications; mean and standard deviation were calculated using MS-Excel. The significant difference of mean values ($P < 0.05$) was analyzed by Two-way Analysis of Variance (ANOVA) followed by Duncan's Post Hoc Test for honest significant difference.

Results and Discussion

The exoskeleton of insects plays a major role during different phases of life cycle. The proximate composition of exuviae of the silkworm *A. mylitta* is given in Table 1. The values obtained for crude protein, chitin, ash, fat, moisture, crude fiber and carbohydrate content from the present study was 62%, 11.52%, 10.57%, 3.9%, 3.1%, 2.5% and 0.9%, respectively (Table 1). Cuticular proteins and chitin are two most important elements of insect exoskeletons, which protect insects from mechanical damage, dehydration, infection and maintain insect body shape (Andersen *et al.,* 1995; Vincent and Wegst, 2004; Guan *et al.,* 2006). Earlier findings suggest that in the exuviae, protein varies between 44 -61%, chitin accounts for a range of 11-42% and fat content was 1-2%, and the variation depends on the type and life cycle phases of species (Kramer *et al.,* 1995).

In the present study, ash content of the exuviae was 10.57%. Further, ICP-OES study confirmed the presence of calcium, magnesium, manganese, zinc, copper and sodium in exuviae (Table 2). Since calcium is the major component (0.4208%), and plays significant role in various intracellular

Table 1: Proximate composition of exuviae of silkworm *Antheraea mylitta* (g 100 g^{-1} d.wt.)

Components	Composition (%)
Protein	61.97 ± 0.02
Fat	3.9 ± 0.08
Chitin	11.52 ± 0.32
Ash	10.57 ± 0.2
Moisture	3.145 ± 0.03
Crude fiber	2.5 ± 0.07
Carbohydrate	0.94 ± 0.04

Values are mean of three replicates±S.D

Table 2: Elemental composition of exuviae of silkworm *Antheraea mylitta* (g 100 g^{-1} d.wt.)

Elements	Availability (%)
Calcium	0.4208
Magnesium	0.113
Manganese	0.007
Copper	0.0055
Sodium	0.0478
Zinc	0.0077

Table 3: LC-MS phenolic acid and flavonoids profiling of exuviae extracts

Phenolic acids	Percentage	Flavonoids	Percentage
Benzoic acid	0.7590±0.01	Umbelliferone	1.86±0.041
p-Hydroxy benzoic acid	0.3074±0.002	Apigenin	0.26±0.004
Salicylic acid	0.2067±0.004	Galangin	0.02±0.001
3-Hydroxy benzoic acid	0.3622±0.007	Naringenin	13.36±0.221
t-Cinnamic acid	0.0150±0.001	Kaemperol	1.68±0.01
2,4-Dihydroxybenzoic acid	0.5173±0.002	Luteolin	52.64±1.3
Gentisic acid	49.3738±0.322	Fisetin	0.0019±0.001
Protocatechuic acid	2.9620±0.03	Eriodictyol	0.001±0.0001
p-Coumaric acid	40.7942±0.14	Catechin	12.95±0.735
o-Coumaric acid	0.4814±0.14	Epicatechin	9.20±0.119
Vanillic acid	0.5382±0.013	Hesperetin	0.58±0.012
Gallic acid	1.4534±0.056	Quercetin	3.65±0.045
Caffeic acid	0.9829±0.11	Epigallocatechin	1.73±0.062
Ferulic acid	1.1464±0.89	Myricetin	1.30±0.067
Syringic acid	0.0257±0.001	Rutin	0.77±0.071
Sinapic acid	0.0737±0.006	**Total**	**100**
Ellagic acid	0.0006±0.000034		
Chlorogenic acid	0.0005±0.00001		
Total	**100**		

Values are mean of three replicates±S.D.

Table 4: LC-MS Phenolic acid and flavonoids profiling of exuviae extracts

	Zone of inhibition (mm^2)						
Bacterial strains	**50µg**	**100µg**	**150µg**	**200µg**	**250µg**	**Tetracyclin (5µg)**	**Methanol**
C. albicans	17.08	146.01	282.6	365.62	523	261.12	0
P. aeruginosa	0	41.1	47.13	53.02	55.02	304.86	0
L. acidophilus	0	4.09	42.58	48.67	71.97	510.59	9.11
S. mutans	41.1	59.94	100.35	146.01	226.08	314.63	0
E. faecalis	0	27.12	39.63	41.01	41.1	372.68	0
B. subtilis	0	0	32.53	47.13	73.75	282.6	0

Data are expressed in % out of total content.

processes, such as neurotransmission, muscle contraction, regulation of enzymatic activities and patterns of gene expression, hormone and neurotransmitter release (Catterall, 2000) and moulting (Fieber and Lutz, 1985). Further, as compared to other exuvial components, fat content was very low (Table 1), prior to pupation, silkworms stop feeding, and use body lipid as an energy source during pupal transformation. Further, the level of fat in the whole pupal body (23.83%) was more than 5-6 times that found in the exuviae. The availability of nutritional proximate suggests that exuviae powder can be used as a cheap source of food.

In the present study, phenolic and flavonoid profiling was analyzed through LC-MS. The polyphenolic components of exuviae were gentisic acid, gallic acid, p-coumaric acid, caffeic acid, protocatechuic acid, and ferulic acid (Table 1). Similarly, the flavonoid profiling indicates the presence of Luteolin, Naringenin, Catechin, Epicatechin, Quercetin, Umbelliferone, Epigallocatechin, and Myricetin (Table 1). Flavonoids and phenolic compounds exhibit an array of biological properties, which include scavenging of free radicals, antiviral, anti-inflammatory, antibacterial and antiallergic actions (Tungmunnithum *et al.,* 2018; Kumar and Pandey, 2013; Chen *et al.,* 2015; Dzialo *et al.,* 2016; Andreu *et al.,* 2018; Meng *et al.,* 2018). In the isolated pigments (Fig. 1), UV-Vis spectra showed one major peak at 230 nm and one minor wide peak at 265-285 nm (Fig. 2). Most of the phenolic and flavonoids compound also absorb light in this range (230-285nm). Based on the UV-Vis absorption maxima, the fluorescent emission spectra of exuviae extract was measured. In comparison to excitation wavelength at 265nm, 230nm showed high fluorescence intensity (emission)at 470nm (Fig. 3). Further, when the extract was exposed to UV lamp, bluish green fluorescence was observed (Fig. 1). Similarly, closure emission spectra (450nm), as well as bluish-green

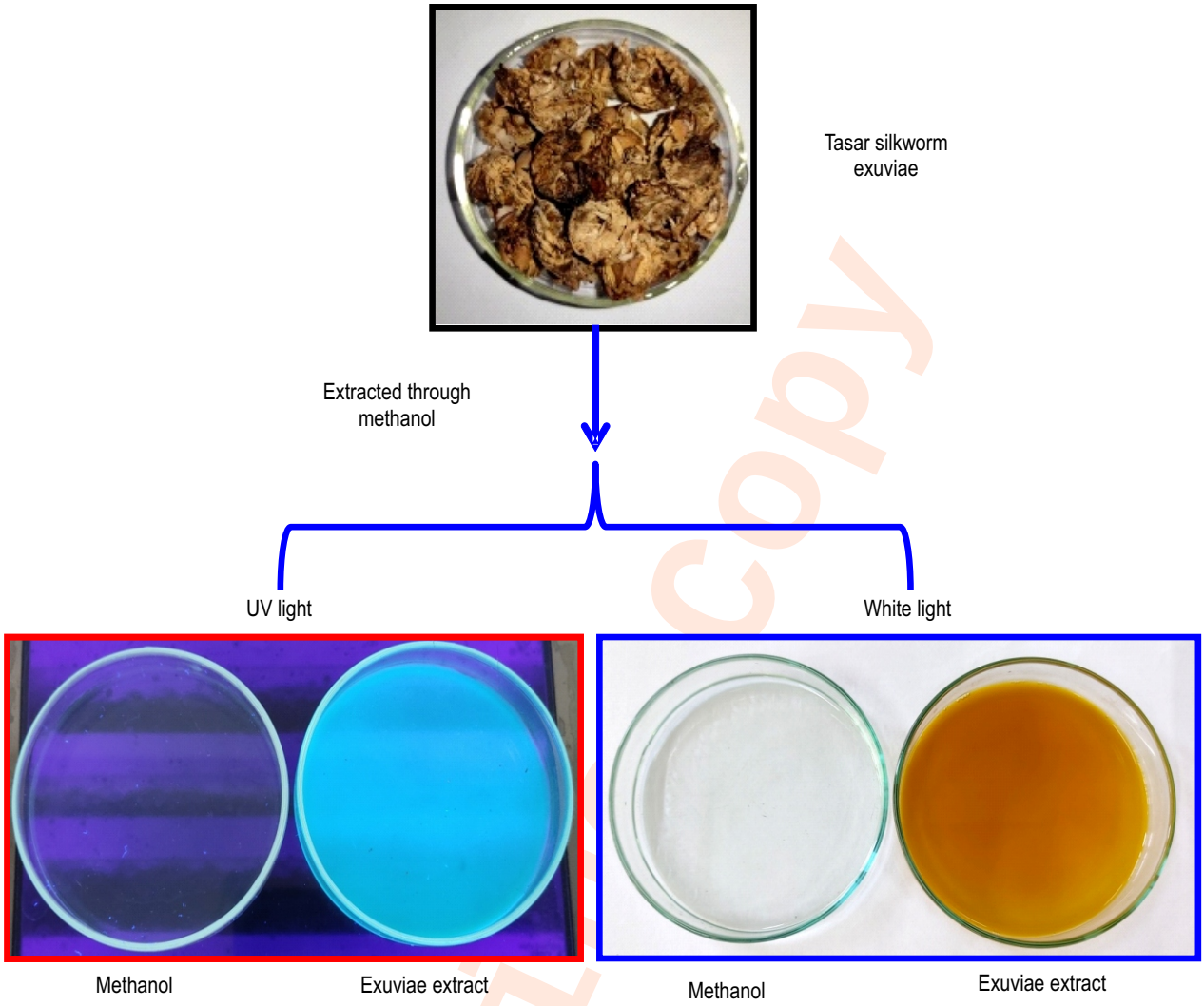

Fig. 1: Comparative optical fluorescence under UV and white light.

fluorescence of silk gland was also observed by Fan *et al.* (2019) in silkworm feeding on carbon nanodots. Further, through LC-MS study it was confirmed that more than one flavonoid and phenolic compounds were present in the extract (Table 1). Phenolic compounds and flavonoids possess many important biological activities (Nijveldt *et al.,* 2001), and the antioxidant potential is considered to be partly related to most of those bioactivities. Therefore, *in-vitro* antioxidant potential is often used to evaluate the properties of an extract. Different *in-vitro* methods are found in literature for the scavenging activity of free radicals, however, in the present study, DPPH, H_2O_2 and NO scavenging potential were adopted to assay such activity of the methanolic extract of exuviae. The biological systems are continuously challenged by free radicals generation which in turn has potential to cause damage to cells and biomolecules through oxidative process leading to manifestation of many disease conditions. Antioxidants play major role to counter oxidative stress mainly through scavenging of free radicals, inhibition of lipid peroxidation and some other mechanisms thereby protecting the cells and tissues from oxidative damages (Pari and Amarnath, 2004). Therefore, we analyzed the antioxidant activities of the methanol extract of exuviae by well accepted standard DPPH assay. DPPH is a free radical, having odd number of electrons shows maximum higher absorption at 517 nm. DPPH becomes paired off consequent to the reaction with antioxidants, and thus gets reduced to DPPH, with a decrease in absorbance (Blois, 1958). In the present study, DPPH radical scavenging assay revealed dose dependent stronger antioxidant properties of the exuviae extract (Fig. 4).

This clearly indicates that the scavenging potential of the extract was positively proportionate with the sample dose ($P<0.05$). Similar observations on free radical scavenging activities of polyphenols and flavonoids are reported by various researchers (Anibarro-Ortega *et al.,* 2020; Dong *et al.,* 2017;

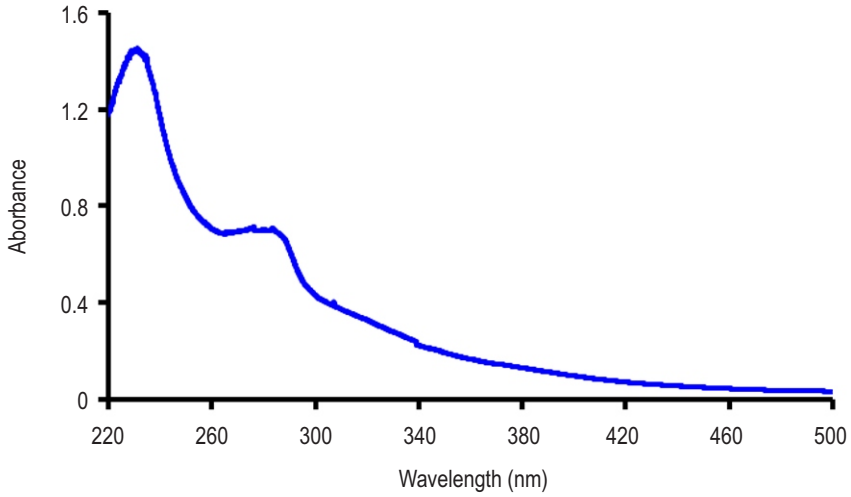

Fig. 2: UV-Vis absorption spectra of exuviae extract.

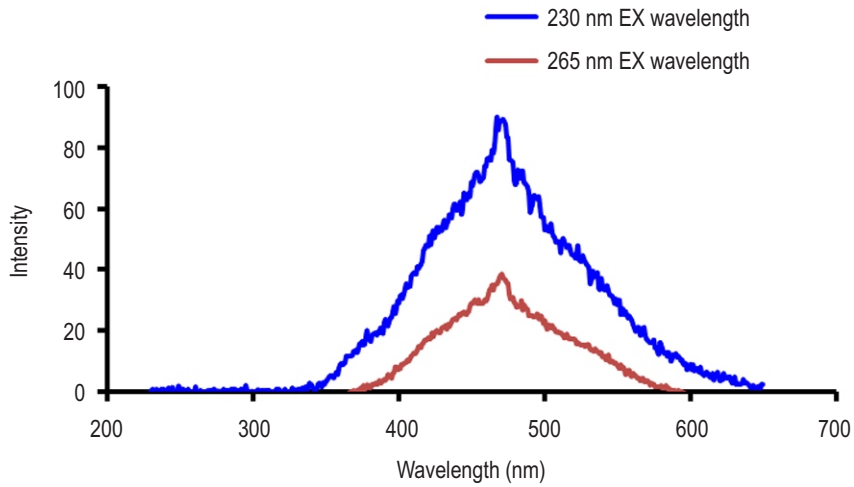

Fig. 3: Emission spectra of exuviae extract at different excitation wavelength.

Mardani Ghahfarokhi and Farhoosh, 2020; Murakami *et al.,* 2015; Rasmi *et al.,* 2018). Further, there exist a positive correlation ($P < 0.001$) between phenolic contents of ME and DPPH potential (Fig. 5), which further substantiates the findings of the present study. The phenolic and flavonoid profiling showed that the exuviae contained gentisic acid, p-coumaric acid, luteolin, naringenin, catechin, epicatechin, and quercetin (Table 3).

Further, DPPH scavenging potential of luteolin (Dong *et al.,* 2017), naringenin (Rashmi *et al.,* 2018), catechin (Choi *et al.,* 2001), gentisic acid (Mardani Ghahfarokhi and Farhoosh, 2020), p-Coumaric acid (Kilic and Yesiloglu, 2013) and quercetin (Murakami *et al.,* 2015) has been reported by several researchers. The DPPH scavenging potential may be due to the occurrence of these polyphenols and flavonoids in the methanolic extract of exuviae. Free radicals are the molecules in the biological system which contain unpaired electrons, making them highly reactive. In order to achieve stable state, they either donate or accept electrons from other surrounding molecules. In this pretext, H_2O_2 is not free radical that belongs among the ROS, but in the presence of metallic ions like Fe^{2+} or Cu^{2+} and superoxide anions, it produces highly reactive hydroxyl radicals under Fenton reaction (Halliwell and Gutteridge, 2001) that lead to lipid peroxidation besides DNA damage. As shown in Fig. 4, the H_2O_2 scavenging potential of ME significantly varied with dose, which may be due to the phenolic and flavonoids compounds that

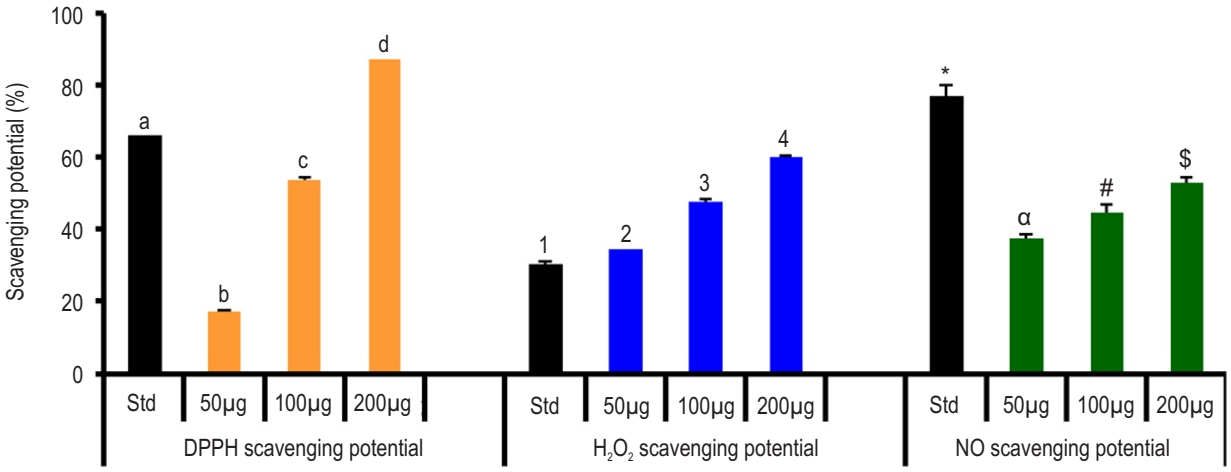

Fig. 4: DPPH scavenging potential, hydrogen peroxide scavenging potential and Nitrous oxide scavenging potential of flurophore (exuvae extracts). Data expressed as mean ± SD (n = 5). Superscripts of different letters are significantly different from each other at P < 0.001.

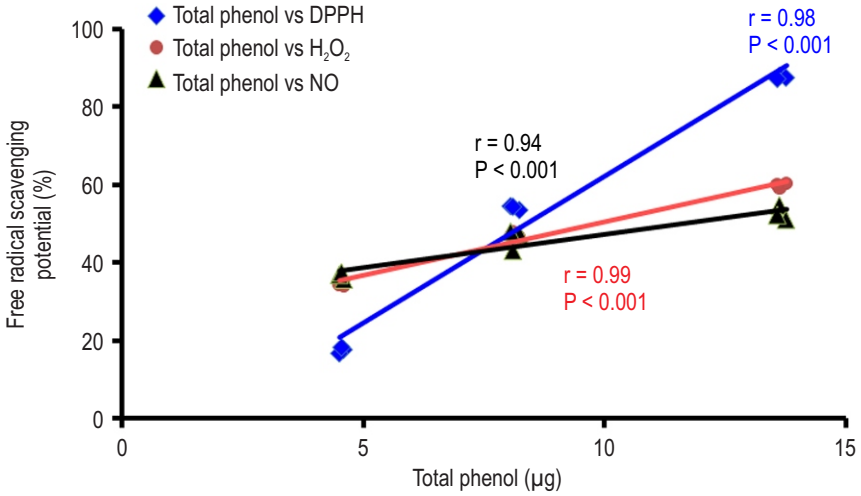

Fig. 5: Scatter plot and correlation curves between Phenolic content vs DPPH scavenging potential. Phenolic content vs H_2O_2 scavenging potential and Phenolic content vs NO scavenging potential.

donate electrons to hydrogen peroxide, thereby neutralizing it into H_2O. Further, in this study the phenolic contents of extract was positively correlated (P <0.001) with the scavenging potential of H_2O_2 (Fig. 5), which provides further supporting evidence. Similarly, antioxidant response of luteolin and quercetin against H_2O_2 in HMB-2 cells was reported by Horvathova *et al.* (2005). Further, the extract sample contains higher quantities of gentisic acid and p-coumaric acid (Table 3). Earlier findings suggest that gentisic acid having peroxide scavenging potential (Chandrasekar *et al.,* 2016a), due to the fact that there was substitution of hydroxyl at 2 and 5 positions of aromatic ring (Chandrasekar *et al.,* 2016b). Similarly, H_2O_2 scavenging potential of p-coumaric acid has been reported by Kilic and Yesiloglu (2013). The current findings suggest that combined action of flavonoids and phenolic may directly scavenge H_2O_2. Further, other experiments confirm that tasar fluorophore (separated from cocoon) show remarkable tolerance to H_2O_2 induced oxidative stress when compared with control.

The author explained fluorophore can either directly scavenge H_2O_2 or modulates endogenous pathways for tolerance of H_2O_2 toxicity (Kusurkar *et al.,* 2015). Nitric oxide is formed from L-arginine by phagocytes, vascular endothelial cells, and certain brain cells. In different physiological processes, it acts as a

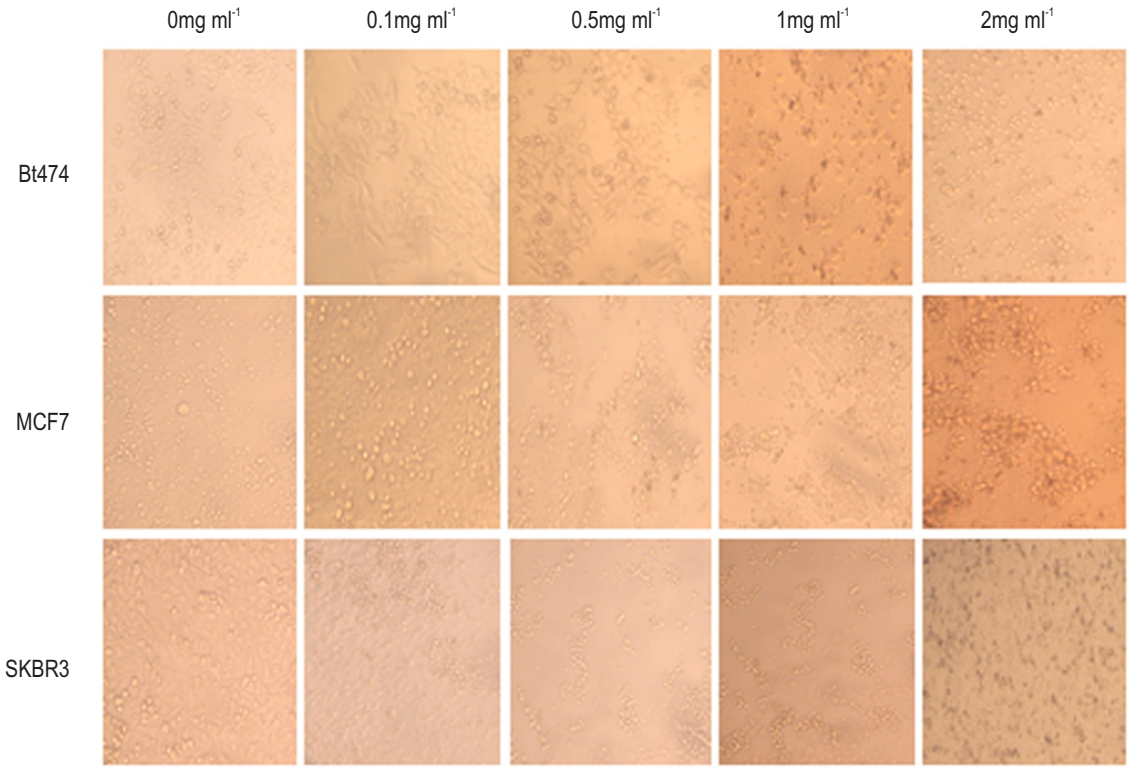

Fig. 6: Morphological modifications of breast cancer cells including BT474, MCF-7 and SKBR3 treated with ME at concentrations of 0.0 mg ml^{-1} (control), 0.1 mg ml^{-1}, 0.5 mg ml^{-1}, 1 mg ml^{-1} and 2.0 mg ml^{-1} after 72 hrs of incubation. (All images are magnified at 20 x).

pleiotropic mediator and effector molecule as it is a diffusible free radical (Miller *et al.,* 1993). Further, NO reacts with superoxide anions and form highly reactive peroxynitrite anion ($ONOO^-$) that causes deleterious effects in the cell. In this study, nitric oxide radical scavenging activity of methanolic extract was found to increase significantly in proportion to the concentration (Fig. 4). Nitric oxide generated from sodium nitroprusside reacts with oxygen and form nitrite. The nitrite ions in turn react with sulphanilamide acid and develop pink colour coupled with naphthyl ethylenediamine and showed maximum absorbance at 546 nm (Panda *et al.,* 2009). As antioxidant (ascorbic acid) donates proton to nitrite radical showing decrease in absorbance which is the basis for estimating nitrite radical scavenging potential of a compound. As the extract contain polyphenols and flavonoids which might donate proton to the nitrite radical and decreased absorbance. Similarly, scavenging potential of flavonoids and phenolics against nitric oxide has been reported by van Acker *et al.* (1995) and Habu and Ibeh, (2015). The anticancer effect of ME in vitro was also explored.

Methanolic extract displayed cytotoxicity to BT474, MCF7 and SKBR3 cell lines (Fig. 6). The WST assay demonstrated inhibition of breast carcinoma cells proliferation in a dose and duration dependent fashion when treated with ME (Fig. 7). This might be due to the presence of phenolics in ME. Phenolic compounds are widely used as components of medicines for the treatment of diabetes, cardiovascular and neurodegenerative and other diseases besides cancer. Their potential actions against proliferation of cancer cells are mainly due to antioxidant activity as they are strong scavengers of free radicals, chelators for different metal ions, regulators of diverse transcription factors and peptides, enhancers of glutathione redox status, modifiers in antioxidant defense mechanisms as superoxide dismutase, catalase and glutathione peroxidases (Ls and Nja, 2016; Chander, 2018). Further, their anticancer effects are associated with the ability in cell proliferation inhibition, angiogenic factors, cascades of oncogenic signaling, induction of apoptosis, and prevention of cellular migration and metastasis (Ls and Nja, 2016; Srinivasulu *et al.,* 2018). Further, it is worth mentioning that gentisic acid, p-coumaric acid, luteolin, naringenin, catechin, and epicatechinis are considered most abundant polyphenols this extract (Table 3) and the anti-proliferative nature against breast cancer cell lines has also been reported earlier Kolahi *et al.,* 2016; Cook, 2018; Zhao *et al.,* 2019; Alshatwi, 2010).

Disc diffusion assay was adopted for antimicrobial activity of fluorescent compounds. Both Gram-negative and Gram-positive bacterial growth was inhibited by pigments (Table 4). The action of potent pigment was found to be bactericidal against

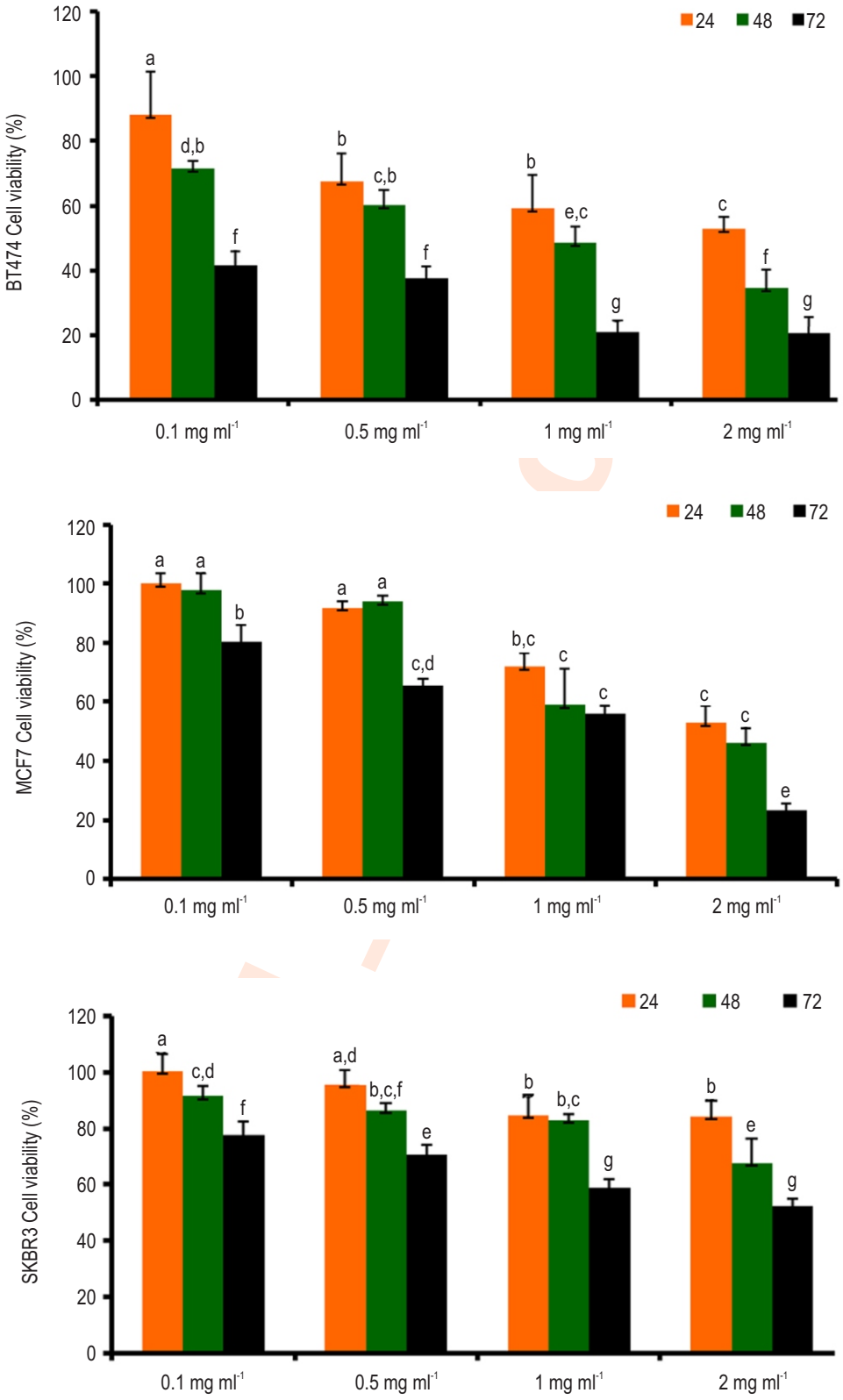

Fig. 7: Cytotoxic potential of flurophore (exuviae extracts) on breast cancer cell line (BT747, MCF7 & SKBR3). Data expressed as mean of three replicates ± SD. Superscripts of different letters are significantly different from each other at $P < 0.05$.

growing cells. Earlier, Xie *et al.* (2015) had mentioned the modes of action as well as mechanisms of selected flavonoids as potent antimicrobial agents. They have demonstrated inhibition in nucleic acid synthesis pathway, alteration in the function of cytoplasmic membranes such as permeability, energy metabolism, and formation of biofilm. In this study, the bactericidal activity seemed to be influenced by phenolic and flavonoids composition and is in accordance with earlier studies the that demonstrate the role of phenolic composition on antimicrobial activity (Shamsudin *et al.*, 2022; Ortega-Vidal *et al.*, 2022). Further, from the phenolic and flavonoid profiling it has been found that the exuviae contains gentisic acid, p-coumaric acid, gallic acid, luteolin, naringenin, catechin, epicatechin, quercetin etc. (Table 3). Further, p-coumaric acid contributes to dual damage mechanisms in order to kill bacteria, first of all by increasing the outer and plasma membrane permeability and secondly inhibiting bacterial cellular functions through binding to genomic DNA (Lou *et al.,* 2012), and also catechin (Diaz-Gomez *et al.,* 2013). In fact, Alvarado-Martinez (2020) demonstrated that gallic acid is an antibacterial molecule that can inhibit bacteria through changes in the outer layer permeability. Other phenolic and flavonoid compounds such as naringenin (Ngun *et al.,* 2015), luteolin (Guo *et al.,* 2020), qurcetin (Jaisinghani, 2017) and epicartechin (Gopal *et al.,* 2016), have antibacterial properties.

This is the first report on tasar silk waste exuviae which gives insight to new scientific information based on the proximal chemical analysis, antioxidant potential, anti-cancer and anti-bacterial potential. The methanolic extract of exuviae showed free radical scavenging potential. Quantitative and qualitative analysis of extracts indicated the presence of phenolic and flavonoids compounds. Moreover, the above data indicate that, exuviae was rich in yellow flavonoids (luteolin, catechin, epicatechin and quercetin) and phenolic acids (p-coumaric acid, gentisic acid and gallic acid), which glows during exposure of UV light. Flavonoids protect against UV radiation, antimicrobial and antiviral in nature may be due to the above properties, wild silkworm *A. mylitta* accumulated selected phenolic and flavonoids on the skin for better adaptability to external environment.

Further, exuviae contains many health promoting polyphenols and glowing antioxidants, anti-microbial and anti-cancer properties, future studies are required for the isolation of the natural products (fluorophore) with fascinating biological and pharmacological properties. Exuviae is a silk waste generated during fiber extraction as well as during large scale tasar egg production. The findings of the present study hold promise to generate additional income leading to socio-economic benefits of farmers as the exuviae has promising pharmaceutical and nutraceutical applications with environmental safety.

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Add-on Information

Authors' contribution: K. Jena: Suggested the study, carried out its design, interpreted the results, data analysis and approved the final manuscript; **S. Ananta**, **Chakrapani and A. Sinha:** Carried out biochemical analysis; **J. Akthar:** Carried out anti-cancer study; **A. Patnaik:** Carried out anti-microbial study; **P.K. Kar**, **J.P. Pandey and S. Kutala:** Language addition, interpretation of the results and data analysis.

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