Exploring Phytochemical Composition and Pharmacological Attributes of *Tamarindus indicia* Seed Coat Extract

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Abstract:

Phytochemicals in the plant are source for new drugs as they have fewer side effects when compared with synthetic drugs. *Tamarindus indica* seed coat is a by-product investigated for its potential and value-added application. Cancer is the deadliest disease ever the society witnessed. Conventional and modern techniques employed for the treatment of cancer such as., chemotherapy, radiation therapy, and surgery, have their own limitations such as cost effective and are not target specific. Oxidative stress exacerbates aging and contributes to various disorders. Plant-derived compounds offer promising avenues for drug discovery, with herbal remedies are often safer and more effective than synthetic drugs. *Tamarind seed* polysaccharides are rich in diverse phytochemicals which are predominantly used in treatment of various aliments they have proven to be very effective antioxidant and anticancer property. The *T. indica* seed coat (TS) extract was extracted using different solvents to determine the phytochemical composition and evaluated for its antioxidant and anticancer properties. In our study the *T. indica* seed coat extracts showed profound antioxidant activity with IC_{50} Value of 160.38 µg/ml. Methanolic extract of *T. indica* seed coat extract showed excellent anticancer activity against A549 cell line it showed IC_{50} Value of 173.62 (µg/ml). Our study paved the way to utilize the *T. indica* seed coat agro waste as a valuable pharmaceutical compound and concludes the potential benefits of *T. indica* seed coat extract to be effective antioxidant and anticancer agent.

Key points: Phytochemical tests, Soxhalet Extraction, Anticancer, Antioxidant activity,

INTRODUCTION:

The Medicinal plants are rich in secondary metabolites, which are the non-nutrient compounds like, alkaloids, flavonoids, saponins and other active metabolites which are of great preventive and therapeutic value and have been extensively used in the drug and pharmaceutical industry. (Singh and Chouhan, 2014). Herbal drug and phytoconstituents resembles safety and efficacy, they produce less no side effect when compared to synthetic drugs. Availability of advanced screening techniques and the demand for phytomedicine initiated the need to investigate, identify, isolate and purify the actual compound of interest. Cancer remains one of the deadliest diseases affecting society, with its mortality rate rising gradually due to excessive chemical exposure and sedentary lifestyles. The increasing prevalence of cancer poses a significant challenge to public health, requiring urgent attention and effective intervention strategies. Techniques such as, chemotherapy, radiation therapy, and surgery are used to treat cancer effectively, however, all the above have some disadvantages (Karpuz, *et al* 2018). There is an urgent need to find out a suitable solution against the cancer.

Free radicals and oxidants released in body have negative effect on the nearby biomolecules. The free radicals and oxidative damage is linked with aging and manifestation of deadliest diseases. Phytochemicals are the rich reservoirs of antioxidants which stop the damage created by free radicals. The healing power of tamarind is first mentioned in the traditional Sanskrit literatures. Several studies have revealed the presence of various phytochemical in *T. indica* with possible antioxidant capabilities. *T. indica* seeds was reported to contain various polyphenolic compounds dominated by proanthocyanidins in the form of catechin, epicatechin, procyanindin dimers, procyanidin tetramers, procyanidin hexamers and flavonoids (taxifolin, apigenin, eriodictyol, luteolin, and naringenin) (Osawa *et al*., 1994; Atawodi, 2012). It has numerous phytochemicals some of which have been reported to possess anti diabetic, antimicrobial, anti-venom, hepatoprotective, and antiasthmatic, laxative and anti hyperlipidemic activities (Bhadoriya *et al.,* 2011). In the present study phytochemical analysis of the *Tamarind indica* seed Coat extract was carried out using different extracts by soxhalet extraction method. The phytochemicals present in the seed coat extract was analysed for its antioxidant activity by DPPH method. MTT assay for anticancer activity was carried out against lung cancer cells.

2. Materials and Methods

1. Collection and Preparation of *T. indica* Seed Coat Extract

T. indica seed coat was extracted mechanically by grinding and kept aseptically in the laboratory. The collected seed coat extract was finely powdered.

2. Extraction Procedure

The coarsely ground sample was subjected for the Soxhlet extraction using solvents of increasing polarity, such as chloroform, ethyl acetate, methanol, and distilled water. The extraction procedure was carried out in a 1:10 ratio, with 50g of dried plant material extracted in 500mL of the respective solvent for a minimum of 5-6 cycles at 37°C.After extraction the respective solvent extracts were dried at room temperature using desiccator. Further the extracts were subjected to preliminary phytochemical screening by performing various biochemical tests. The extracts obtained using solvents were concentrated using rotary vacuum evaporator and then dried. The extract thus obtained was used for various analyses.

3. Phytochemical Screening

Phytochemical screening of *T. indica* seed Coat extracts were carried out using Chloroform, Methanol, Acetone and Distilled water as a solvent. The preliminary phytochemical analysis was performed using standard procedures. The *T. indica* seed Coat extracts obtained were subjected to preliminary phytochemical screening by performing following chemical tests.

a) Test for alkaloids

i) **Wagner's Test:** To identify alkaloids in *Tamarind indica* seed coat extract, 2-3ml of extract was treated with few drops of Wagner's reagent, formation of reddish brown precipitate indicated the presence of alkaloids.

ii) **Dragendorff's Test:** To detect alkaloids *Tamarind indica* seed coat extract, 2-3 mL of extract was treated with few drops of Dragendorff's reagent, formation of an orange-brown precipitate indicated the presence of alkaloids.

a) **Test for Flavonoids:** To detect flavonoids in *T. indica* seed coat extract, few drops of 1% Ferric chloride solution were added; the formation of blackish red colour indicated the presence of flavonoids. (Senguttuvan *et al.,* 2014).

b) **Test for Glycosides by Keller-Kiliani :** To detect glycosides in *T. indica* seed coat extract, 2 mL of the extract was mixed with glacial acetic acid, followed by one drop of 5% $FeCl_3$ and concentrated H_2SO_4. The formation of reddish brown color at the junction of the two liquid layers and upper layer appears bluish green confirmed the presence of glycosides in the sample.

c) **Test for Phenols by** $FeCl_3$ test: To identify phenols in *T. indica* seed coat extract, 5% $FeCl_3$ solution was added. Appearance of deep blue- black color indicated the presence of phenols

(Ren *et a*l., 2009).

d) **Detection of Saponins by Foam test:** The *T. indica* seed Coat extract was diluted with 20ml of distilled water and it was shaken in a graduated cylinder for 15min.The formation of a 1cm foam layer confirmed the presence of saponins.

e) **Test for Tannins by Ferric chloride**: *Tamarind indica* seed Coat extract was mixed with liquid gelatin and the formation of a white precipitate confirmed the presence of tannins.

f) **Detection of Terpenoids by Salkowski test:** To 2ml of *T. indica* seed Coat extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added and shake well. Formation of reddish brown color confirmed the presence of terpeniods.

4. Quantitative Analysis of Bioactive Compounds

a) Estimation of Total Phenolic Content (Folin-Ciocalteu Reagent Test):

The total phenolic content of methanol and distilled water extract of *Tamarind indica* seed coat was determined using the Folin Ciocalteu method (Selvin *et al.,* 2020). Total phenolic content was calculated based on a calibration curve of gallic acid and expressed in terms of Gallic Acid Equivalents (GAE) per gram of extracts.

b) **Estimation of Total flavonoid content**: The Flavanoid content in the *Tamarind indica* seed coat was detected by colorimetric assay aluminum chloride was used to obtain the total flavonoid content (Zhishen *et al.,* 1999). Standard Quercetin concentrations of 20–100 µg/mL were prepared in methanol for calibration.

5. Antioxidant Activity

a) DPPH assay: The Free radical scavenging activity of *T.indica* seed coat extract was assessed using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The extract was combined in various concentrations with 1ml of DPPH solution, shaken vigorously and allowed to stand for 30 min in the dark chamber. Absorbance was measured at 517 nm against a blank. IC_{50} value was determined by interpolation from linear regression analysis. Ascorbic acid was used as standard for comparison studies. The capacity of radical scavenging activity was calculated using the following equation (Rice-evans *et al.,* 1997).

$$\textbf{DPPH scavenging effect (\%)} = [\{A_0 - A_1\}] \times 100$$

Whereas, A_0 is the absorbance of the control reaction and A_1 is the absorbance of the sample.

6. Cytotoxicity Assay

a) Anticancer activity by MTT Assay

The MTT assay is used to evaluate cell proliferation rate and a reduction in cell viability indicates apoptosis or necrosis (Gerlier *et al., 1986*). Anticancer activity of *Tamarind indica* seed coat extract was assessed different concentrations (50, 100, 150, 200 & 250 µg/ml) for sample from stock. Test compound were added to the respective wells and after incubation, the drug-containing media was aspirated.100µl of medium containing 10% MTT reagent was added to each well to get a final concentration of 0.5mg/ml and the plate was incubated at 37ºC and 5% CO_2 atmosphere for 3 h. Absorbance was measured at 570 nm and 630 nm using microplate reader. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line (Alley *et al*.,1986).

The percentage of cell viability and inhibition was calculated using the following equations:

Percent cell viability = (Average absorbance of treated cells /Average absorbance of untreated cells) × 100

Percent cell inhibition = 100 - percent cell viability

The net absorbance from the control wells was taken as 100 percent viable

3. Result and Discussion

3.1 Collection and Preparation of *T. indica* Seed Coat Extract

The *Tamarindus* fruit was collected from Jhamakhandi Vijayapura and Dharwad. The seeds were extracted from fruit aseptically. The seeds were sun dried and crushed finely to form a coarse powder (Fig.1). The coarse powder of seed coat was subjected to Soxhalet extraction to detect the presence of phytochemicals by using different solvents such as chloroform, Ethyl acetate, Methanol and Distilled water (Table-1).

Fig 1: a) *T. indica* Seeds b) *T. indica* Seed Coat extract

Table: 1 phytochemical analysis of *T. indica* seed coat extract

Tests	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	Negative	Negative	Negative	positive
Flavonoids	Negative	Negative	positive	positive
Glycosides	positive	Negative	Negative	Negative
Phenols	Negative	Negative	positive	positive
Saponins	Negative	Negative	Negative	positive
Tannins	Negative	Negative	positive	positive
Terpenoids	Negative	Negative	positive	positive
Steroids	positive	Positive	Negative	positive

3.2 Phytochemical Analysis Results:

Phytochemical analysis results showed that among the selected *T. indica* seed coat extracts there is strong variation in the presence of secondary metabolites. Glycosides was present in chloroform and ethyl acetate extracts whereas absent in Methanolic and aqueous extract. Methanolic and distilled water extract showed the presence of flavonoids, alkaloids, phenols and Terpenoids as major phytochemicals and distilled water extract have showed the presence of flavonoids, alkaloids, phenols, Terpenoids and steroids.

3.3 **Quantitative Analysis of Bioactive Compounds**

a) Estimation of Total Phenolic Content

Total phenolic content of Methanolic extract of *T. indica* seed coat found to be 341.88µg/g whereas Aqueous extract of *T. indica* seed coat showed 188.94µg/g.

b) Estimation of Total flavonoid content

The flavonoid content of Methanolic extract of *T. indica* seed coat is 3829.76µg/g whereas the flavonoid content of Aqueous extract of *T. indica* seed coat showed 2765.32µg/g respectively

3.4 Antioxidant Activity

i) **Antioxidant activity of aqueous extract of tamarind seed coat**

Free radicals are highly reactive molecule and responsible for so many diseases like cancer and cardiovascular disease. Antioxidants are endogenous or exogenous molecules responsible for scavenging of free radicals. The raw extracts or the chemical constituents derived from *T. indica* seed coat extract are effective in scavenging the free radicals and inhibiting the destructive processes induced by oxidative stress. In our study the Aqueous

extract of *Tamarind* Seed Coat at 250 µg /ml showed maximum cell inhibition and IC_{50} value of 160.38 µg /ml.

ii) Antioxidant activity of Methanolic extract of tamarind seed coat

Oxidative damages induced by free radicals have devastating effect on normal health and paved a way to numerous diseases and also accelerate aging process. Imbalance in anti-oxidants leads to oxidative stress, and induces cell injury and death. Moreover, most of these drugs are highly expensive, mutagenic, and carcinogenic. Now day's researchers focused on the use of natural antioxidants to inhibit lipid peroxidation, to protect the damage caused by free radicals. *T. indica* seed coat extract restores reactive oxygen species by changing the glutathione level and antioxidant enzyme expression in response to oxidative stress. The Methanollic extract of *T. indica Seed* Coat at 250 µg /ml showed maximum cell inhibition and IC_{50} value of 125 µg /ml(Fig.2).

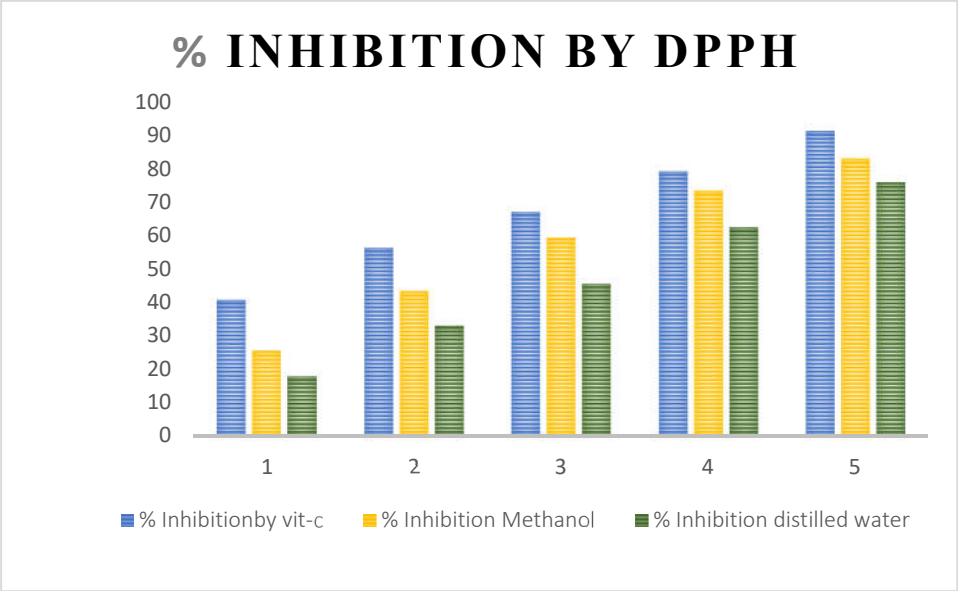

Fig 2: Antioxidant activity of aqueous extract of *T. indica* seed coat extract

3.5 Anticancer activity by MTT Assay

Anticancer activity of *T. indica* seed coat extract was carried out at different concentrations (50, 100, 150, 200 & 250 µg/ml for sample from stock) of test drugs were added to the respective wells. Methanolic extract of *T. indica* seed coat extract at 250 µg/g showed excellent anticancer activity against A 549 cell line it showed IC_{50} Value of 173.62 (µg/ml) (Fig.3&4).

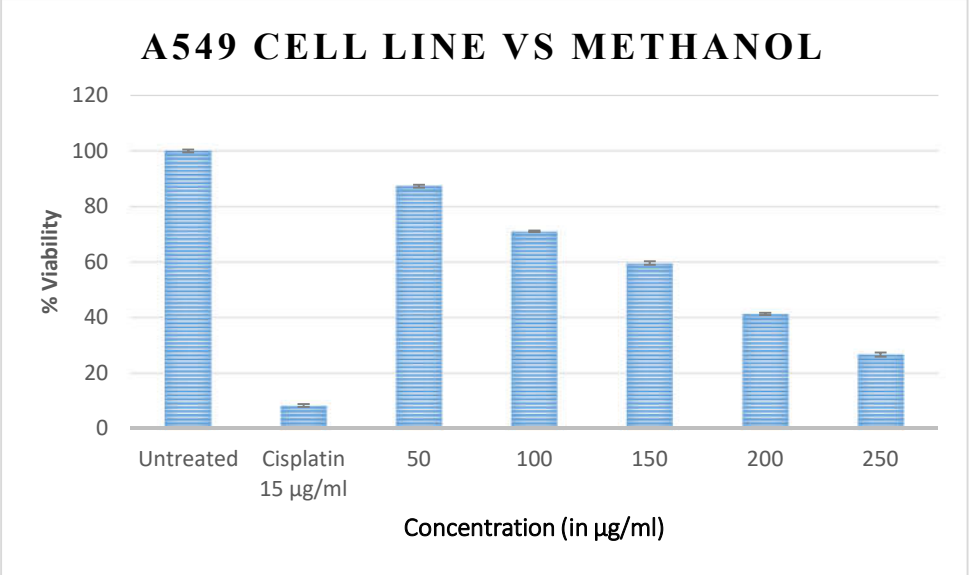

Fig.3 Anti-cancer activity of *T. indica* Seed coat extract

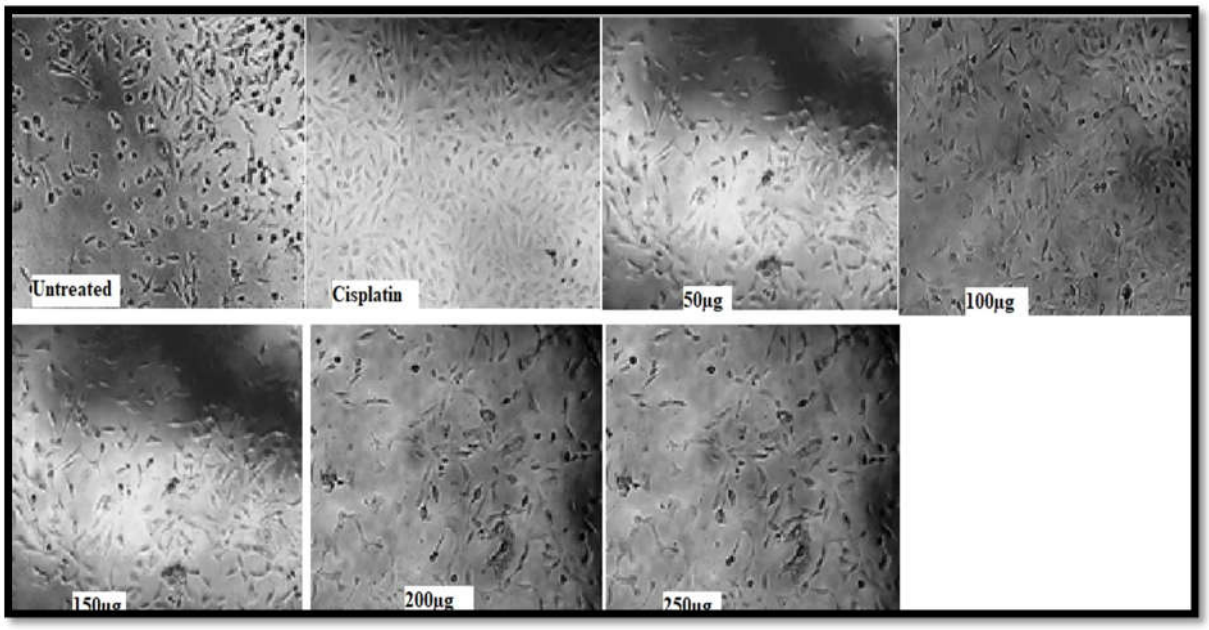

Fig.4 Anticancer activity of Methanolic extract of *T. indica* seed coat at 50 µg to 250 µg

Discussion:

Plants have significantly impacted on human society from olden days due to their rich repository for diverse phytochemicals, and these plant bioactive compounds have been widely used to treat various diseases. Phytochemicals present in the plants play a pivotal role in physiological process such as reproduction, pollination and in their defence against harsh conditions like drought, UV radiation, high temperature and pollution (Xie *et al.,* 2019).

Alkaloids are known to play biological roles and control the development in living system. Methanol extract of *T. indica* seed coat extract showed the presence of flavonoids, alkaloids, phenols and terpenoids as major phytochemicals whereas in the aqueous extract *T. indica* seed coat extract showed the presence of flavonoids, alkaloids, phenols, terpenoids and steroids. respectively. In our study the aqueous extract of the *T. indica* seed showed the presence of Alkaloids. Methanollic and aqueous extract of *T. indica* seed coat extract showed the presence of flavonoids. Polysaccharide isolated from tamarind seeds has biological applications. It has immunomodulatory effect and lacks carcinogenic and cytotoxic activities (Sreelekha *et al*., 1993; Sano et al., 1996; Iida *et al.,* 1978) Wandee, R et al 2022 in their study report the total phenolic content as 106.40 0.69 mg Gallic acid equivalence/g extract, and total flavonoid content was 0.45 0.07 mg Quercetin was used as standard drug. In the present study the total flavonoid content of methanolic extract of *T. indica* seed coat extract found to be 3829.76µg/g, whereas the Aqueous extract of *T. indica* seed coat extract was 2765.32µg/g. Total phenolic content of methanolic and aqueous extract of *T. indica* seed coat extract was found to be 341.88µg/g, and 188.94µg/g. Glycosides also have vast therapeutic efficacy and they are found in almost all medicinal plants. Study by Yadav *et al* the confirmed the presence of tannins in extracts of leaves and seed coat of *T. indica*. (Yadav *et al.,* 2014). The qualitative phytochemical analysis conducted by Sandesh *et al* 2022 on seed coat of T.indica showed the presence of glycoside, diterpenes, phenolic, steroids, tannins, flavonoids and saponins. Our study correlates with the study conducted by Sandesh. The anti-oxidative activity of tamarind seed was also investigated by Osawa *et al.* (1994, in El-Siddig *et al*., 2006). They found that ethanol and ethyl acetate extracts prepared from the seed coat exhibited anti-oxidative activity. Anti-oxidative property of *T. indica* seed coat extract was proven by the studies conducted by Nakchat *et al.* 2014; Sandesh *et al.* 2014; Suksomtip *et al*. 2010. Leaf extracts exhibit antioxidant activity in the liver (El-Siddig et al., 2006). Antioxidant activity of tamarind leaves reported by Perez *et al.* (2003) and Ramos *et al.* (2003). The *T. indica* seed coat found to be an excellent antioxidant due to its components, such as , phenolic, procyanidins, flavonoids and tannin which have reported to possesses antioxidant by elevated the non-enzymatic and enzymatic antioxidant system (Ameeramja et al. 2016; Nakchat et al. 2014a, 2014b).Procyanidin, catechin, rutin and embelin have been revealed to be effective anti-oxidant and anti-inflammatory properties, which are capable of inhibiting oxidative stress. (Babu 2008). The methanolic and aqueous extract of *T. indica* seed coat extract was analysed for Antioxidant activity by H_2O_2 and DPPH method. Antioxidant activity of Aqueous extract of *T. indica* seed coat extract by H_2O_2 method showed IC_{50} Value of 73.74 µg/ml. Aqueous extract

of *T. indica* seed coat extract showed IC_{50} Value of 160.38 µg/ml. by DPPH method wherein Ascorbic acid was used as a standard. The existence of catechins polymerized forms and derivatives in the TS extract has been presumed; this has been suggested from the demonstration of a potent ant oxidative activity of the TS extract over the catechin standard compound which has agreed with previous findings of Liang, *et al*. 2016.

Anticancer activity of *T. indica* seed coat extract was carried out by MTT assay against A549 cell using cisplatin as a standard drug. Sandesh *et al* 2022, performed anticancer activity of methanolic extract againast MCF-7 cancer cell line and found IC_{50} value of 16 µg/mL. Methanolic extract of *T. indica* seed coat extract showed excellent anticancer activity against A549 cell line as it showed IC_{50} Value of 173.62 (µg/ml). Flavonoids possess free radical scavenging effect and therefore inhibit tumour invasion and metastasis (Komori *et al.,* 1993; Tanaka *et al.,* 1997). Flavonoids were determined to have *in vivo* activity on experimental tumours. The phytochemicals present in *Tamarind Indica* seed coat extract effectively used as an anticancer and antioxidant agent.

Conclusions: In our study the by-product *T. indica* seed coat was effectively explored as the valuable resources as a pharmaceutical product for the Circular economy and sustainable ecosystem. The tamarind seed coat as a by-product attracted the researcher's interest due to its high content of phytochemicals which can be used as a potent source for production of antioxidant and anticancer agents. *Tamarind Indica* seed coat extract is a by-product from tamarind fruit is have limited application in pharma field but our study successfully highlighted the importance of phytochemicals present in seed coat.

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