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A REVIEW ON ANTI-OXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITES OF CAESALPINIA SAPPAN BARK.

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Abstract:

In many occasions, human beings are exposed to various environmental, occupational and xenobiotics challenges due to modern life styles. Enormous free radicals are generated during exposure to suck stressful conditions. In addition to this, the process of metabolism and excretion of xenobiotics may also generate free radicals. Thus, generated free radicals may produce beneficial oxidation thereby releases energy. However, the excessive free radicals may attack DNA, cell membranes and other cellular components and destroy them. The inbuilt free radicals scavenging system like glutathione, SOD, catalase etc., are involved in scavenging of the excessive free radicals. But the continuous exposure to the stressful conditions may be overpowering the inbuilt scavenging capacity of the cells leading to cellular destruction. The various free radicals that are released in to the body are superoxide anion (O_2), NO radical NOO., OH. And H_2O_2 radical. Thus, released free radicals react with the membrane polyunsaturated lipids and oxidize them to lipid peroxides. This lipid peroxidation damage membrane protein as well as the lipids and thereby the integrity of membrane is lost. Therefore, it is considered that the extent of lipid peroxidation is directly proportional to cell damage. In addition, the free radicals may also attack DNA and causes tissue damage.

Hyperlipidaemia is the greatest risk factor contributing to atherosclerosis & occurrence of coronary heart disease & cerebrovascular accidents. Hence hypolipidemic drugs are extensively used as prophylactic agents to prevent such atherosclerosis induced disorders. plants have been documented to have significant hypolipidemic actions. Based on these phytoconstituents the present is focused on review on antioxidant and antihyperlipidemic activities of *Caesalpinia sappan* bark.

Key Words: *Caesalpinia sappan*, flavonoids, Anti-oxidant, Anti- hyperlipidaemic.

INTRODUCTION:

The GATT implementation in India many of the pharmaceutical companies were come-forward to establish natural isolated compounds to made it as a lead compound. The recent systemic research has been carried out on bio compounds have been used for many disorders and diseases treatment. These natural obtaining compounds have less side effects as compared to synthetic analogues. The 17% of rural people depends on natural drugs, by considering all these facts the research has been focused on using *Caesalpinia sappan (CS*) plant material, from which therapeutical active constituents were extracted and obtained extracts were subjected for antioxidant and antihyperlipidemic activities on experimental tools. The *Caesalpinia sappan (CS*) bark containing rich constituent of flavonoids and other phytoconstituents. hence the investigation was targeted on antioxidant and antihyperlipidemic activity was performed using DPPH method and triton induced method respectively. Our body is exposed to a large number of foreign chemicals every day. The most of which are manmade and our inability to properly metabolize of free radicals, free radicals are also generated during normal metabolize of aerobic cells. The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumours and coronary heart disease. The various reactive species include superoxide ions, hydrogen peroxide, and hydroxyl, Nitric oxide, per oxynitrate radicals, which play a significant role in oxidative stress related to the pathogenesis of various important diseases. These species cause the cellular damage by reacting with various lipids, enzymes, and nucleic acids. This damage is the major contributor of the production of free radicals in healthy individuals and is balanced by the antioxidative defence system. The present investigation was focused on antioxidant and antihyperlipidemic activities in animals.

REVIEW OF LITERATURE

1. K Harjit, *et al.*,(2016) conducted a study on Evaluation of Antioxidant and Anthelmintic Properties of *Caesalpinia sappan L*. Leaves. The aim of this research was to study the qualitative phytochemistry and to determine the antioxidant and anthelmintic activities of *Caesalpinia sappan* leaves. Phytochemical analysis indicated the presence of various plant bioactive metabolites in Caesalpinia *sappan* leaves. Different solvent extracts of the crude drug was tested for in vitro antioxidant potential using 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay and ferric ion-reducing antioxidant power (FRAP) assay.Results of this study revealed that *Caesalpinia sappan* leaves methanolic extract has significant antioxidant potential as compared with standard, vitamin C. Methanol extract also exhibited potent anthelmintic activity with paralysis time (19.13±0.340 min) and death time (54.21±0.533 min). The result of present study shows that *Caesalpinia sappan* leaves can serve as a good natural source of potent antioxidants and anthelminthiasis in preventing oxidative stress and helminthiasis. [2]

2. Shrishailappa B, *et al.*, (2003) conducted a study on Antioxidant Activity of *Caesalpinia sappan* Heartwood. It was studied both by in vitro and in vivo models. The antioxidant activity of the plant extract

and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The ethyl acetate, methanol and water extracts exhibited strong antioxidant activity as evidenced by the low IC50 values in both 1,1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide methods. The values were found to be less or comparable to those of ascorbic acid and rutin, the standards used. Administration of the successive methanol and water extracts at 50 and 100 mg/kg body weight given for four days prior to carbon tetrachloride (CCl_4) treatment caused a significant increase in the level of superoxide dismutase (SOD) and catalase and a significant decrease in the level of thiobarbituric acid reactive substances (TBARS), when compared to CCl_4 treated control in both liver and kidney. These changes observed at 100 mg/kg body weight treatment were comparable to those observed for standard vitamin E at 50 mg/kg treatment. The results support significant antioxidant nature of *Caesalpinia sappan* heartwood extracts. [3]

3. Yuan Li, *et al.*, (2021) conducted research on Activity Screening of the Herb *Caesalpinia sappan* and an Analysis of Its Antitumor Effects. In this research, the antitumor activity and mechanisms of the leaves and stems were compared with the roots of *Caesalpinia sappan*; it was in order to investigate whether stems and leaves of *Caesalpinia sappan* could be used to replace heartwood for antitumor treatment. MTT assays were used to identify the active sites of Caesalpinia sappan based on the application of human liver cancer (HuH-7) cells. High-performance liquid chromatography (HPLC) was used to analyse polar extracts. The result shows IC50 for the petroleum ether extract of roots was 56.10 µg/ml, while that for the leaves and stems was 77.20 µg/ml. Grey relational analysis indicated 11 active fraction peaks that were closely related to antitumor activity. This data indicates that the mechanisms underlying these effects may relate to a reduction in the expression of PCNA and VEGF and the inhibition of angiogenesis. [4]

4. V Sathya Srilakshmi, *et al.*, (2010) performed a study on Hepatoprotective properties of *Caesalpinia sappan* Linn. heartwood on carbon tetrachloride induced toxicity. Aim of the study was to investigate the methanol and aqueous extracts of heartwood of *Caesalpinia sappan* for its hepatoprotective activity against CCl_4 induced toxicity in freshly isolated rat hepatocytes and animals. They are exposed to CCl_4 (1%) along with/without various concentrations of methanolic and aqueous extract of *Caesalpinia sappan* (1000-800 µg/ml) and the levels of selected liver enzymes were estimated and Antihepatotoxic effect of methanolic extract was observed. Wistar strain albino rat model was used for the investigation of in vivo hepatoprotective properties. Liver damage was induced by ip administration of CCl_4 (30%) suspended in olive oil (1 ml/kg body weight). Both the tested extracts showed potent hepatoprotective activity at 200 mg/kg body weight test dose which was comparable with that of the standard silymarin used in similar test dose. The methanolic and aqueous extract was able to restore the biochemical levels to normal which were altered due to CCl_4 intoxication in freshly isolated rat hepatocytes and also in animals. [5]

5. Nirmal N, et al., (2014) studied on Wound healing activity of standardized brazilin rich extract from *Caesalpinia sappan* heartwood. The aim of this study was to evaluate the wound healing effect of standardized brazilin rich extract (BRE). In vitro cell proliferation and migration of human fibroblast cell were determined by using scratch wound assay technique. Cytotoxicity, antioxidant and antibacterial activity of brazilin and BRE were also evaluated. Result indicated that brazilin and BRE had showed similar antioxidant and

antibacterial activity (P > 0.05). Cytotoxicity study revealed that brazilin or BRE was nontoxic up to 500 µg/mL concentrations. Invitro scratch wound assay showed that BRE at 250 µg/ mL had highest cell proliferation and migration on day 2 of incubation. BRE treatment resulted in restoration of original cell number within 2 days when compared to control (P< 0.05). This study reveals brazilin rich extract (BRE) could be used as a replacer for brazilin owing to its similar biological activities and ease of low-cost production. [6]

6. Nyi Mekar Saptarini, et al., (2021) conducted a study on Analgesic and Antipyretic Activities of Ethanolic Extract of Sappan wood (*Caesalpinia sappan* L.) Leaves. The aim of this study was to determine the analgesic and antipyretic activity of ethanolic extract of sapan wood leaves in Webster mice as experimental animals. To determine the analgesic activity in acetic acid-induced mice with mefenamic acid as a positive control, the writhing method was used. The temperature reduction method was used to determine the antipyretic activity in yeast-induced mice with paracetamol as a positive control. One-way ANOVA was conducted for statistical analysis, followed by Tukey Kramer post hoc test. Phytochemical screening showed that sappan wood contains alkaloids, flavonoids, saponins, monoterpenoids, and sesquiterpenoids. The optimum dose of analgesic and antipyretic activity was 6.3 mg and 8.4 mg/20 g BW of mice, respectively. The result shows ethanolic extract of sappan wood leaves has analgesic and antipyretic activities. [7]

7. Chia-Hua Liang, et al., (2013) conducted research on Brazilein from *Caesalpinia sappan* L. Antioxidant Inhibits Adipocyte Differentiation and Induces Apoptosis through Caspase-3 Activity and Anthelmintic Activities against Hymenolepis nana and Anisakis simplex. s. This study verifies the antioxidant and antitumor characteristics of brazilein in skin cancer cells and is the first time to elucidate the inhibition mechanism of adipocyte differentiation, cestocidal activities against Hymenolepis nana, and reduction of spontaneous movement in Anisakis simplex. The free radical scavenging activities of brazilein were determined by DPPH· and ABTS·+ scavenging assays. The toxic effects of brazilein were evaluated in terms of cell viability, induction of apoptosis, and the activity of caspase-3 in BCC cells. The inhibition of lipid peroxidation by brazilein (5, 10, 20, 30, 40, 50, and 100 μM) was determined by using
the liposome as a model of biological membranes. The results are expressed as mean ± standard deviation (SD). Statistical differences were estimated by one-way analysis of variance (ANOVA) followed by Dunnett's test or the Tukey-Kramer test. These results reveal that brazilein, a purified substance from *Caesalpinia sappan* L., scavenges free radicals and inhibits lipid peroxidation, indicating antioxidation activity.[8]

8. Eui-Gil Jung,et al., (2015) conducted a study on Brazilin isolated from *Caesalpinia sappan* L. inhibits rheumatoid arthritis activity in a type-II collagen induced arthritis mouse model. The aim of this study was to understand the anti-rheumatoid activity of brazilin that was isolated from ethyl acetate extract of C. sappan L. The evaluations were conducted in mice with type-II collagen-induced arthritis (CIA). Brazilin was purified via preparative HPLC and identified by mass spectrometry and 1H/13C NMR analysis. DBA/1J mice were divided into four groups (n = 10). Three groups of mice received intradermal injections of inducer bovine type-II collagen (BTIIC; 2 mg/ml in 0.05 ml acetic acid) and 0.1 ml of booster complete Freund's adjuvant (CFA). A second injection of BTIIC with booster incomplete Freund's adjuvant (ICFA) was given

subsequently after 21 days. On 22nd day, purified brazilin (10 mg/kg body weight) or the disease-modifying anti-rheumatic drug methotrexate (3 mg/kg body weight) was administered intraperitoneally daily or every three days for 21 days, respectively to two groups of mice. At the 42nd day, mice sera were collected, and the levels of pro inflammatory cytokines and stress enzyme markers in serum were measured using standard immunoassay methods. The microstructure and morphometric analyses of the bones were assessed using high-resolution microfocal computed tomography. Result shows Brazilin isolated from *Caesalpinia sappan* reduced the arthritis index score and the extent of acute inflammatory paw edema in CIA-mice. Result indicates that brazilin prepared from ethyl acetate extracts of *Caesalpinia sappan* effectively reduced the serum levels of TNF-α, IL-1β, and IL-6; maintained the bone surface pattern; and decreased both paw swelling in the CIA mice model. These results suggest that brazilin derived from the heartwood of *Caesalpinia sappan* may be useful to treat both RA and other inflammatory disorders. [9]

9. Yan-Jun Wan, et al., (2019) conducted a study on The Ethanolic Extract of *Caesalpinia sappan* Heartwood Inhibits Cerebral Ischemia/Reperfusion Injury in a Rat Model Through a Multi-Targeted Pharmacological Mechanism. In this study, a middle cerebral artery occlusion (MCAO) rat model was employed to elucidate the mechanism of the anti-cerebral ischemic effects of *Caesalpinia sappan* ethanolic extract (CEE). Systemic multi-target identification coupled with gene ontology biological process (GO BP) and reactome pathway analysis was used to investigate the potential neuroprotective mechanism. The presumed mechanism was confirmed through biological analysis by determining the effects of CEE on the identified signalling pathways in PC12 cells model-induced by oxygen-glucose deprivation/reperfusion
(OGD/R). This study demonstrates that CEE (both through in vivo administration at a dosage of 300 mg/kg and through in vitro incubation at a dosage of 2.4 μg/mL) is a neuroprotective agent that can effectively inhibit neuronal damage, promote synaptic generation, and suppress the activation of neutrophils, microglia, and astrocytes. This study demonstrates that CEE can exert neuroprotective effects through the regulation of neuronal apoptosis, neuroinflammation and axonal generation to prevent ischemia/reperfusion-induced cerebral injury. [10]

10. Shengqian Q Wu,et al.,(2012) conducted a study on Anti-inflammatory activity of an ethanolic *Caesalpinia sappan* extract in human chondrocytes and macrophages. Statistics were performed using one-way analysis of variance (ANOVA) with post hoc Tukey tests, cross-comparing all study groups (95% confidence interval). Primary human chondrocytes were isolated from cartilage specimens of osteo arthritis (OA) patients. Primary cells, SW1353 chondrocytes and THP-1 macrophages were serum starved and pre-treated with different concentrations of CSE. Following viability tests, nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) were evaluated by Griess assay and ELISA, respectively. Using validated real-time PCR assays, mRNA levels of IL-1ß, TNF-α, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) were quantified. SW1353 cells were cotransfected with a COX-2 luciferase reporter plasmid and nuclear factor-kappa-B (NF-κB) p50 and p65 expression vectors in the presence or absence of CSE. Results showed that CSE dose-dependently inhibited the expression of pro-inflammatory cytokines IL-1ß and TNF-α in IL-1ß-stimulated

chondrocytes and LPS-stimulated THP-1 macrophages. CSE further suppressed the synthesis of NO in primary OA chondrocytes by blocking iNOS mRNA expression. This report demonstrates the anti-inflammatory activity of CSE in an in vitro cell model of joint inflammation. Blockade of IL-1ß-induced NF-κB signaling and its downstream pro-inflammatory targets by CSE may be beneficial for reducing cartilage breakdown in arthritis. Using human OA chondrocytes and THP-1 macrophages, the present study demonstrates that CSE is a potent inhibitor of proinflammatory mediators in the context of joint inflammation. [11]

11. Irmanida Batubara, et al., (2009) studied about Brazilin from *Caesalpinia sappan* wood as an antiacne agent. The separation of the extract components was performed by column chromatography and preparative high-performance liquid chromatography (HPLC). Brazilin, protosappanin A, and sappanone B were isolated from methanolic extracts. Brazilin showed better antibacterial activity [minimum inhibitory concentration (MIC)= minimum bactericidal concentration (MBC) = 0.50 mg/ml] than protosappanin A (MIC = MBC = 1.00 mg/ ml) and sappanone B (MIC = MBC > 2.00 mg/ml). The 50% inhibitory concentration (IC50) for lipase inhibition was lowest for brazilin (6 µM), which showed strong inhibition compared with protosappanin A (100 µM) and chloramphenicol (677 µM, positive control). The antioxidant activity of brazilin and protosappanin A were higher than sappanone B (IC50 14.5 µM). This study demonstrates that Brazilin is considered to have sufficiently potent activity for use as an antiacne agent. [12]

12.Tran Manh hung, et al.,(2013) conducted a study on Cytotoxic Activity of New Phenolic Compounds from Vietnamese *Caesalpinia sappan*. Two new phenolic compounds, caesalpiniaphenols G– H (1 and 2), were isolated from Vietnamese Caesalpinia sappan heartwood. The chemical structures were established mainly by extensive spectroscopic studies and chemical evidence. Compounds 1 and 2 showed potent inhibitory activity against HL-60 cancer cell lines with respective IC50 values of 16.7 and 22.5g/mL. Treating HL-60 cells with various concentrations of 1 resulted in growth inhibition and the induction of apoptosis. The induction of apoptosis in cancer cells is one of the most useful strategies for anticancer drug development. This study suggested that the active constituents from Vietnamese *Caesalpinia sappan* may be an important source for developing anti-cancer drugs.[13]

13. Sari Haryanti, et al.,(2018) conducted a study on evaluating Cytotoxic and MMPs inhibitory activities of Sappan Wood (*Caesalpinia sappan* L.) in various extracts on 4T1 breast cancer cell line. Sappan wood powder were divided into 4 parts, each part was extracted with different solvent. Each powder was macerated using ethanol 96%, ethanol 70%, and methanol for 3x24 hours. The water extract was done using hot infusion method for 15 minutes. MTT assay is used to identify cytotoxicity effect, and gelatin zymography assay to detect the activity of MMP-2 and MMP-9. Phytochemical profiling of the extract were observed by Thin Layer Chromatography (TLC). The results of MTT assay showed that ethanolic 96% extract exhibited the strongest cytotoxic effect against 4T1 with the IC50 value 13,1 µg/mL, followed by methanolic (21,4 µg/mL), ethanolic 70% (22,5 µg/mL) and water (25,5 µg/mL). The analysis of gelatin zymograph bands using ImageJ software proved that all extracts except water, inhibited gelatinolytic activity of MMP-9. The results in the

present study suggest that various extract of the sappan wood with polar solvent have been found to possess cytotoxic and MMP-9 inhibitory activities and may potentially be explored as antibreast cancer. [14]

14.Liwayway H,et al.,(2019) conducted a study on Sappan (*Caesalpinia Sappan*) Seeds in the Control of Cockroach (Periplenata Americana) . This research was undertaken to determine what concentration of seed ethanol extract (SSEE) will control cockroach. Result of this study is beneficial to rural and urban areas where cockroaches are abundant. It is most beneficial in places where Sappan seeds are just thrown away, which can be used as potential source of insecticide from plants. Experimental research method with four treatments and 80 experimental animals was used. SSEE was macerated in 95% ethanol. Eighty cockroaches were assigned in four groups/treatments, with 20 cockroaches per treatment. They were exposed to different concentrations as; T- 0% SSEE, T1, 25% SSEE, T2, 50% SSEE, and T3, 75% SSEE.

Gathered data was analyzed using Analysis of Variance (ANOVA) and Fisher Least Significant Difference test (LSD). Findings of the study revealed that highest percentage of mortality after 48 hours observation, was obtained from treatment three (75 %percent SSEE). Based on this result Sappan seeds ethanol extract has a significant potential in the control of cockroaches specifically at higher concentration.[15]

15.K. Hemalatha, et al, (2008) conducted a study on Analgesic Activity of *Caesalpinia sappan* Heartwood. The ethanol extract of heartwood of *Caesalpinia sappan* Linn (Caesalpiniaceae) and three crude fractions [petroleum ether (60–80C), diethyl ether, and ethyl acetate] were subjected to preliminary qualitative chemical investigation. The ethanol extract and its fractions underwent pharmacological screening for analgesic activity by using acetic acid-induced writhing in albino mice. All data are expressed as Mean SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnet's test. The ethanol extract and its different fractions (petroleum ether, diethyl ether, and ethyl acetate) of *Caesalpinia sappan* heartwood were found to contain flavonoids, triterpenoids, tannins, and sterols. This present study demonstrates oral administration of the ethanol extract and its different fractions of heartwood of *Caesalpinia sappan* at both dose levels (200 and 400 mg=kg) significantly inhibited acetic acid-induced writhing comparable to the effects of aspirin, indicating that the ethanol extract as well as its fractions had the potential to block the process of pain pathway. In conclusion, the ethanol extract of heartwood of *Caesalpinia sappan* and its three different crude fractions were found to show peripheral analgesic activity.[16]

16.Nam-ln Baek,et.al., (2000) conducted a study on Anticonvulsant Compounds from the Wood of *Caesalpinia sappan* L. 80% Aqueous MeOH extracts from the wood of Caesalpinia sappan, which showed remarkable anticonvulsant activity, were fractionated using EtOAc, n-BuOH, and I-t20. Among them, the EtOAc fraction significantly inhibited the activities of two GABA degradative enzymes, succinic semialdehyde dehydrogenase (SSADH) and succinic semialdehyde reductase (SSAR). Repeated column chromatography for the fraction guided by activity test led to the isolation of the two active principal components. Their chemical structures were determined to be sappanchalcone and brazilin based on spectral data. The pure compounds, sappanchalcone (1) and brazilin (2), inactivated the SSAR activities in a dose dependent manner, whereas SSADH was inhibited partially by sappan chalcone and not by brazilin.[17]

17.Te-Sheng Chang 1, et al., (2012) conducted a study on Melanogenesis Inhibition by Homo isoflavone Sappanone A from *Caesalpinia sappan*. Homo isoflavone, sappanone A, was isolated from *Caesalpinia*

sappan and proven to dose-dependently inhibit both melanogenesis and cellular tyrosinase activity via repressing tyrosinase gene expression in mouse B16 melanoma cells. To our knowledge, sappanone A is the first homo isoflavone to be discovered with melanogenesis inhibitory activity. Our results give a new impetus to the future search for other homo isoflavone melanogenesis inhibitors. In conclusion, our results not only demonstrate that sappanone A is the first homo isoflavone to be discovered with melanogenesis inhibitory activity, but also give a new impetus to the future search for another homo isoflavone melanogenesis inhibitors.[18]

18.K.Mekala, et al.,(2016) conducted a study on Herbal Formulation development for Hypolipidemic and Anti-Obesity activity on Heartwood of *Caesalpinia sappan* Linn. The hydro alcoholic extract (1:1) on heartwood of *Caesalpinia sappan* was prepared by cold maceration method and formulated into a hard gelatine herbal capsule. The quantitative analysis of phytoconstituents especially of flavonoids and phenolic content present both in extract and formulated herbal capsule was estimated by HPLC method showed the presence of rutin, quercetin, gallic acid, ascorbic acid and tannic acid. Hydroalcoholic extract of Caesalpinia sappan (HECS) herbal capsule was evaluated for acute toxicity studies in albino Wister rats and showed no toxicity up to 2000 mg/kg. Inivo evaluation of high fat diet (HFD) induced obesity in rats was carried out for HECS herbal capsule. The studies showed HECS herbal capsule (200 mg/kg and 400 mg/kg) significantly reduced the elevated levels of body weight, total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, SGPT and SGOT and elevated the decrease level of HDL-cholesterol. These results suggest that, HECS capsule possess good hypolipidemic and anti-obesity activity, which may be due to its flavonoid, saponin and phenolic content.[19]

19.Sunitha V S, et al., (2014) conducted a study on Immunomodulatory Activity of *Caesalpinia sappan* L. Extracts on Peritoneal Macrophage of Albino Mice. The present study was conducted to scientifically evaluate the effects of extracts of Caesalpinia sappan on phagocytic function of macrophages. In vivo effect of aqueous, ethanol and hexane extract of the plant at two doses (10 mg/kg body weight and 25 mg/kg b.w.) were evaluated by oral administration of the extracts on Swiss albino male mice. In vitro immunomodulatory potential of the above extracts at different concentrations (10µg/ml, 25µg/ml, 50 µg/ml and 100µg/ml) was studied using peritoneal macrophages from Swiss albino mice. All extracts gave phagocytic modulation in vivo. The ethanol extract of *Caesalpinia sappan* at a dose of 25 mg/kg b.w. showed significant ($p<0.05$) increase in phagocytic activity in comparison with the control. An increased phagocytic response was shown by murine peritoneal macrophages after treatment with the extracts in vitro. A dose dependent response was observed in all cases. The results of the present study indicate the immunomodulatory effect of *Caesalpinia sappan* extracts on murine peritoneal macrophages, as evidenced by its effect on phagocytosis which is a nonspecific immune mechanism.[20]

20. Helmi, et al., (2020) conducted a study on *Caesalpinia sappan* L. Wood is a Potential Source of Natural Phosphodiesterase-1 Inhibitors. The ethanol extract of CS wood and its fractions were evaluated in vitro by using a cyclic nucleotide phosphodiesterase assay kit. The presence of brazilin in the extract and fractions was analysed by thin layer chromatography. In silico assay was performed using MOE software to obtain insights into the interaction between compounds in the CS wood and the enzyme. The results shows that Ethanol

extract and ethyl acetate soluble fraction effectively inhibited the PDE1 activity. Interestingly, brazilin, the major compound in CS wood, also exhibited a potent inhibitory effect on the enzyme. The in-silico assay revealed that the interaction between tetraacetylbrazilin and brazilin with the PDE1 B active site involved hydrogen bonding and π–π interactions. [21]

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