Over the past decade, herbal medicine has attracted increasing interest from the psychiatry research community for its better compliance and lower side effects. All selected plants (*Dalbergia sissoo* Roxb., *Citrus limon* Linn. and *Elaeocarpus sphaericus*) were commonly used in traditional medicine, but no scientific work has been conducted on the anxiolytic activity of the bark. The results from the studies confirmed that the methanol stem bark extract of *Dalbergia sissoo* Roxb. possesses a strong anxiolytic potential. By column chromatography, four compounds were isolated which were further evaluated for anxiolytic activity with two renowned models and neurotransmitter studies, that prove compound A and compound-B (both has basic moiety neoflavonoid) exactly responsible for anxiolytic activity in methanol stem bark extract of *Dalbergia sissoo* Roxb. That effect is due to compound-A alone or in combination with compound-B gives anxiolytic effect. Our study confirmed the possibility that the behavioral effects of flavonoids in anxiety models were through increasing the 5-HT concentrations in hippocampus without causing any sedative effect. The behavioral and transmitter changes observed in the current models confirm that Compound-A can be an effective herbal candidate to treat anxiety mainly through its serotonergic activation. Taken together, we speculate that the serotonergic action of flavonoids might be similar to that of SSRIs; however, investigations on the presumptive adaptive changes such as hippocampal serotonin receptors are still needed to elucidate the precise role of flavonoids and further pharmacological and biochemical investigations are underway to find out exact molecular mechanisms underlying its effects and to better understand the neuropathological changes in anxiety. Therefore, we advance the suggestion that this Compound-A may fulfill the therapeutic need for the treatment of anxiety and related neuropsychiatric disorders. This result with methanolic stem bark extract of *Dalbergia sissoo* Roxb. validate the ethnopharmacological claims.